

**Distribution and factors affecting the abundance
of *Amphibola crenata*
in the Avon-Heathcote Estuary
2008 - 2010**

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Nursalwa Baharuddin

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ABSTRACT

The Avon-Heathcote Estuary is the largest semi-enclosed shallow estuary in Canterbury, New Zealand and functions as a sink for local industrial and domestic treated sewage. The endemic mudflat snail, *Amphibola crenata* is one of the conspicuous members of the estuarine benthos and it is likely to be a good indicator of changes in contaminant and nutrient conditions within the estuary.

This thesis investigated the current densities, distribution, and biomass of *A. crenata* in the Avon-Heathcote Estuary. *A. crenata* was widely distributed throughout the estuary. However, no mudsnails were found in samples collected from southern sites close to the causeway and none were present on the sandbanks in the centre of the estuary. There was a positive correlation between *A. crenata* density and biomass. As the distance from the edge of the estuary and salinity increased, the density and biomass of *A. crenata* decreased. All of the statistical models showed that mudsnails were found close to the estuary edge.

Densities of *A. crenata* were higher in summer compared to winter. Juveniles inhabited the high-tide level while medium and large *A. crenata* were common at the mid-tide and low-tide levels. There was a good correlation between the dry weight and shell length of *A. crenata* for all sites and seasons.

Over a six week period, field cage experiments found that shell growth of adult, medium and juvenile *A. crenata* varied amongst sites based on combination factors including food quality, contaminant levels and sediment conditions. The wet weight of large adults did not change significantly, however, there were differences in weight gain of medium and juveniles from the different sites. Mudsnail condition varied with body size and site. Zinc was the trace metal found in the highest concentrations in the sediment while cadmium was the lowest trace metals detected. Levels of total phosphorus, nitrogen, ammoniacal, kjeldahl nitrogen and dissolved reactive phosphorus in the water were highest at the Oxidation Pond site which was close to the main discharge point from the treatment plant into the Avon-Heathcote Estuary.

A. crenata populations in the present research have shown strong correlations with environmental variables and this can be used for management

and conservation. The research presented in this thesis provides a starting point in our understanding of the effects and implications of diverting treated waste water on the distribution and abundance of a key gastropod. Future research could also include other indicator species and other estuaries and bays of the New Zealand coastline.

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Chapter 1: GENERAL INTRODUCTION

1.1 Estuaries

Estuaries are places where rivers meet the sea. Steffenson (1974) explained that estuaries are an important link in marine, freshwater and terrestrial ecosystems. Pritchard (1967) defined estuaries as semi-enclosed coastal bodies of water which have a free connection with the open sea and within which sea water is measurably diluted with fresh water from land drainage. However, the definition by Pritchard did not include blind or closed estuaries as found in arid countries such as southern Africa and western and southern Australia. Day (1981) defined blind or closed estuaries as those that are situated at the sea mouth and are enclosed by a sand bar for a short period. For example, Malaga Estuary, Kwazulu Natal, in South Africa is closed for most of the year by a sand bar, but after the rainy season, it floods and breaches the barrier, hence lowering the water level as it flows to the sea (Blaber 1997). One of the most usual definitions is that of Day (1980), who defined an estuary as a “partially enclosed coastal body of water which is either permanently or periodically open to the sea, and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage”. The interaction with the sea allows the estuaries to be influenced by tides. The area will be flooded during the incoming tide and emptied on the outgoing or ebbing tide, exposing mud or sand flats. As a result, salinities are variable from ~30 ppt on an incoming tide and 4 ppt on an outgoing tide (Angel 1974; Day 1981). Caspers (1967) defined estuaries as saline areas situated at river mouths in tidal seas; however, estuaries may differ in the amount of tidal influence and fresh water inflow.

According to Dyer (1979) and McClusky (1981), there are three types of estuaries: positive, negative and neutral. In a positive estuary, the volume of fresh water from river and land drainage flowing into the estuary is higher than the evaporation; this is the most common type of the estuary. A negative estuary is where evaporation is higher than the freshwater runoff into the estuary, and these

are most typical in tropical countries. A neutral estuary is when evaporation and the input of freshwater are almost equal. This situation almost never occurs (Dyer 1979; McClusky 1981).

In New Zealand, estuaries are classified into five classes according to their geological history (Hume and Herdendorf 1988). These are formed by (a) fluvial erosion, (b) marine/fluvial erosion, (c) tectonic activity, (d) volcanic activity and (e) glacial erosion. The typical estuary formed by fluvial erosion is funnel-shaped and enclosed by a headland or barrier with a river mouth. Fluvial erosion estuaries were formed when the sea level was lower than at present and river action caused the original depositional basin to be cut. A rise in sea level caused the landform to be drowned and become modified by sediment deposition of both fluvial and marine origin, e.g. Waikopoua and Whangaroa in the North Island of New Zealand. The type of estuary that is formed by marine/fluvial activity is a coastal embayment. This estuary is developed by a combination of factors such as sub-aerial weathering, stream erosion and wave attack. It has a small catchment and very little fluvial input. Examples include Matai, Bland and Mimiwhangata Bays in the North Island of New Zealand. Estuaries are formed by tectonic origin can be categorised into two main types: (a) fault defined embayments and (b) diastrophic embayments. This type of estuary is very rare, but can be found in Hawke and Tasman Bays in New Zealand. When volcanic craters are drowned, breached by the sea and partially infilled with sediment, volcanic embayments are formed, e.g. Orakei and Panmure Basins. Estuaries which are carved by glaciers are very deep, long and narrow. Circulation is restricted due to the sills situated at the entrance and marked stratification of fresh water. Examples of this last type can be found in the western South Island of New Zealand (Hume 2003). The type of Avon-Heathcote Estuary is fluvial erosion and is the largest semi-enclosed shallow estuary in Canterbury and remains one of New Zealand's most important coastal wetlands.

According to Day (1981), estuaries support a wide range of habitats, including rocky outcrops, sandy beaches, mangroves, salt marshes and sub-tidal to intertidal areas. It is an environment that lies between the land and the sea, and

thus can be influenced by fluctuating environmental conditions. Hume and Herdendorf (1988) described estuaries as places that are influenced by oceanic processes, catchment use and in-estuary use. Estuaries have a high variability in abiotic conditions such as salinity, dissolved oxygen, temperature, light, tidal cycles and sediment stability (Kromkamp and Forster 2006). They can be classified as having a stressful physico-chemical environment created by the tidal fluctuations.

Estuaries are very rich in nutrients, especially nitrogen and phosphate, which are supplied directly from rivers, the sea and the land. McClusky (1981) cites an example of the Ythan Estuary in Scotland. Here, the river supplies 70% of the nitrate and 80% of the silicate, whilst the marine environment supplies 70% of the phosphate. Admiraal (1984), found that nitrates, phosphates and ammonia were generally high in estuarine water. Silicate, which is often a common limiting nutrient to diatom communities, was also high. The continuous input from the land, rivers and sea to ecosystems makes estuaries biologically rich and diverse (Admiraal 1984).

Estuaries are one of the most productive habitats worldwide (McClay 1976). However, compared with other aquatic systems, estuaries have a lower diversity of flora and fauna (Dyer 1973). According to McClusky (1981), such conditions arise due to mixing of freshwater and salt, which challenge the fauna to adapt physiologically. Despite a stressful and challenging environment, many flora and fauna such as phytoplankton, microphytobenthos, macroalgae, sea grasses, invertebrates, molluscs, crustaceans, fish and birds can adapt to this changeable environment (Day 1981; Knox and Kilner 1973; Owen 1992; Jones and Marsden 2005). The estuarine environment provides shelter and nursery ground for juvenile fish fauna (Kromkamp and Forster 2006).

Estuarine organisms can be classified into three physiological types which are (a) oligohaline, (b) euryhaline and (c) stenohaline. Oligohaline organisms do not tolerate salinities greater than 0.1 ppt; these are the freshwater organisms. Euryhaline organisms tolerate a wide range of salinities (2–25 ppt) and form the majority of organisms in an estuary. For example, the adult *Amphibola crenata* are

air-breathing euryhaline animals which live in brackish water and occupy a transitional habitat between the marine and terrestrial environments (Watters 1964; Pilkington and Pilkington 1982). Stenohaline organisms tolerate salinities as high as 25 ppt and can be found at the mouth of the estuary. Migrant organisms like birds and fish live temporarily in the estuary before they move from fresh water to the sea or vice versa (McClusky 1981).

Worldwide, estuaries have been exploited and are subjected to a high degree of human interference (Kaiser *et al.* 2005). The level of disturbance includes agricultural, industrial and urban pollutants such as organic matter, and heavy metals contributed by rivers, sewage outfalls and storm drains. Estuaries are also developed into marinas, seaports, industrial parks, cities and garbage dumps (Castro and Huber 2003).

1.2 Estuarine contaminants

In the past, estuaries supported various activities such as main transport routes and natural harbours, and provided major food sources such as fishes and shellfish. Many towns have developed around estuaries due to the usefulness of this ecosystem. According to Stewart (2005), large populations can be found at near or on coastal areas. In New Zealand, 12% of estuaries have greater than 5000 population (McClay 1976). Sources of contamination in estuaries come from industrial and metropolitan areas near estuaries, and point and non-point sources upstream (Chapman and Wang 2001). Non-point pollution comes from run off from agriculture, including pasture; septic tanks and depositions of sewage over a water surface (Carpenter *et al.* 1998). Point pollution comes from discharge industrial waste, wastewater effluent, run-off and infiltration from animal feedlots (Carpenter *et al.* 1998). According to Pereira *et al.* (1988), organic contaminants from industrial effluents are released into the coastal inlets and estuaries where dilution of the ocean minimises the hazardous effects of organic compounds. Vehicle and road densities in the past and present have been the main sources of trace metals such as Cu, Pb and Zn (Hopley *et al.* 1980). The marine environment

faces more pressure with the increase in human population since last century (Meyer-Reil and Koster 2000).

Estuaries act as a sink for contaminants and sediments. For example, once heavy metals are released into the ocean, adsorption of trace metals occurs onto suspended particulate matter and this is followed by sedimentation, which transfers the trace metals into the sediments (Pereira *et al.* 1988). The organic contaminants can be carried into the bottom of the ocean where mixing and dilution occurs by tidal action (Kennish 1992).

The mouth of an estuary consists of coarser sediments, while finer sediments can be found up the estuary due to deposition of sediments by the river (Robb 1988). Fine-grained sediments contain higher concentrations of metal compared to coarse sediments (Zhang *et al.* 2001). Quartz sand can be found in the large grain fraction of sediments and this is a diluting factor for metals (Chang *et al.* 2007). Finer particles have a greater surface area and hence have a high concentration of metal, for example Fe and Mn oxyhydroxides (Birch and Taylor 2000). The capacity of sediment to retain trace metals differs according to each sediment type. For example, in Japan and central Europe, high concentrations of toxic metals from anthropogenic input have increased (Nriagu and Pacyna 1988). Even though contamination by heavy metals may cease, the metals can continue to be released. Cundy *et al.* (2003) gives an example in Bilbao Estuary, Spain, where stringent environmental policies have been applied in reducing the amount of metals into the estuary. However, this estuary is recognised as one of the most polluted estuaries in northern Spain.

According to Kennish (1992), anthropogenic activities have increased worldwide, putting more pressure on the estuarine environment and coastal zone. Estuaries have become modified and altered to support increasing human activities (Robertson *et al.* 2002). We need to understand the ecological impacts of contaminants within these ecosystems in order to manage the coastal marine environment (Morrisey *et al.* 1996).

1.3 Trace metals

Trace metals are metals that are present in animal and plant cells and tissues in small quantities. According to Rainbow (1993), “trace metal” is synonymous with “heavy metal”. In this study, the word trace metal will be used. Trace metals are important for good nutrition; however, higher or excessive quantities can have toxic effects. Generally, trace metals can be found in low concentrations, yet they can have many biological effects (Rainbow 1993). Examples of trace metals include iron, zinc, copper, magnesium, lithium, chromium, nickel, cobalt, arsenic, vanadium, molybdenum and selenium. Copper and zinc are necessary for an organism’s metabolism in low concentrations; however, metals such as cadmium, lead and mercury are less important (Kennish 1992). Animals replace trace metals by consuming plants which have absorbed nutrients such as copper and zinc from the sediment. Higher concentrations or excessive amounts of trace metals are toxic and affect feeding activity, growth, reproduction, behaviour, respiration, osmoregulation or disease resistance (McClusky 1981; Marsden and Wong 2001). Trace metals persist in the environment and can accumulate in animal tissue and pass through the food chain, which is an issue of public concern. (Kennish 1992).

Trace metals are found in the sediment of estuarine ecosystem, which functions as a sink (Meade and Parker 1985). Deposition of trace metals is determined by factors such as the biological, physical, chemical and anthropogenic processes present or active in the estuary (Deely 1991). Metals that have been released into estuaries through anthropogenic input have contributed the same or greater amounts compared to natural weathering processes (Robb 1988). Some metals are not readily broken down because they have high affinity with the sediments (Nipper *et al.* 1997). Estuaries and coastal sediments that are near to the industrial and urban areas are likely to contain high concentrations of trace metals in the sediments and this can affect the organisms within the ecosystem (Achyuthan and Richardmohan 2002). Trace metals have increased in the sediment of San Antonio Bay since pre-industrial times about 100 years BP (Trefry and Presley 1976). In fact, archaeologists and other researchers are able to

measure changes in the level of anthropogenic input in estuaries and coastal zones by examining strata within sediment cores (Erlenkeuser *et al.* 1974; Szefer *et al.* 1995).

Sources of trace metals include point source industrial areas such as boatyards, tanneries and mines (Mills and Williamson 1999). Other anthropogenic inputs include lead from vehicle exhaust, zinc from galvanised plumbing and copper from brake linings (Mills and Williamson 1999). Stormwater can also be contaminated with trace metals such as zinc, lead and copper due to urban activities (Stewart 2005). For example, the main sources of metal contamination in Pearl Estuary, China, are industrial wastewater, domestic sewage, run-off from mining sites upstream and marine traffic (Zhou *et al.* 2004).

Rosalez–Hoz *et al.* (2003) suggested that the effluent discharged from an industrial area into the estuary affect coastal developments as a whole. They gave the example of Coatzacoalcos Estuary, Mexico, where the percentage of metals was higher in the sediments as a result of industrialisation in the vicinity of the Coatzacoalcos River. Cameron (1970) concluded that pollutants may harm bottom-living organisms due to insufficiency of oxygen, which is used by organic matter when it decays.

1.4 Study rationale

In 2009, the Christchurch City Council constructed an ocean pipeline to take the city's treated water from a pumping station at the Wastewater Treatment Plant and transport it by underground pipe three kilometres out into the ocean instead of discharging it into the Avon–Heathcote Estuary (Figure 1.1). The wastewater diversion will result in the loss of a major source of organic matter for primary production (the basis of the food web) (Walrond 2007), so changes in the estuary's ecosystem will be expected.

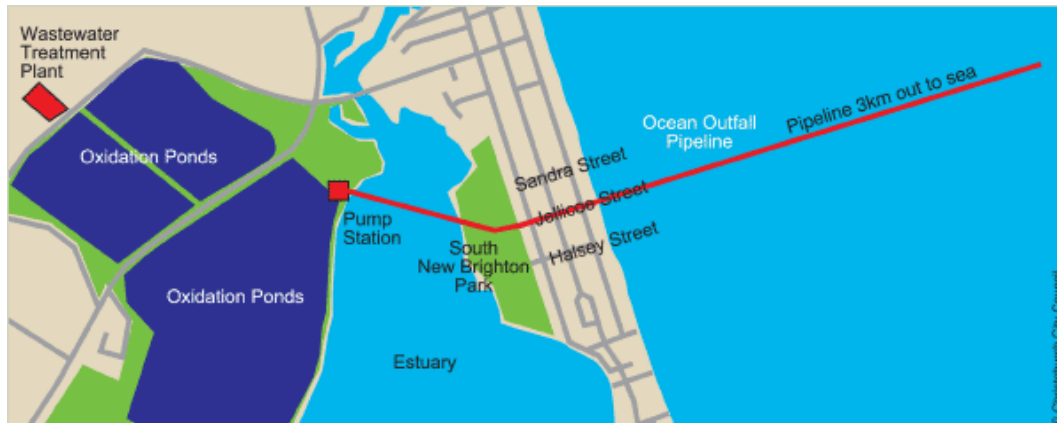


Figure 1.1 Map showing the diversion of treated waste water from the estuary to the ocean along an outfall pipeline. Source Christchurch City Council 2010.

This study will evaluate the current *Amphibola crenata* population in the estuary prior to diversion, in order to allow detection of any post-diversion changes in the Avon-Heathcote estuary. *A. crenata* is endemic to New Zealand and commonly found in all intertidal estuaries (Jones and Marsden 2005). It is an ecological indicator species which is expected to respond to changes in nutrient conditions and eutrophication in terms of density, distribution and population structures.

1.5 Objectives

The primary objectives of this study were to:

- 1) Quantify and assess distribution, densities and biomass of *A. crenata* throughout the Avon-Heathcote Estuary;
- 2) Relate the distribution and abundance of *A. crenata* in relation to its physical and chemical variables such as between seasons, salinity, tidal height, percentage of total organic content and percentage of pore water;
- 3) Experimentally investigate the relationship between the growth rate and condition index of *A. crenata* in relation to food sources; and
- 4) Measure changes in nutrient levels (total nitrogen, phosphorus and trace metals) in the sediment and water.

Chapter 2: FIELD SURVEY

2.1 Study area - The Avon-Heathcote Estuary

The Avon-Heathcote Estuary is a bar-built estuary formed behind a sand spit made up of sediments from the Waimakariri River being transported southwards by long-shore currents (Knox and Kilner 1973). The estuary is located on the eastern side of the city of Christchurch, Canterbury, New Zealand and north of Banks Peninsula at a latitude 43°33' south and a longitude 172°44' east (Knox and Kilner 1973). The Avon-Heathcote Estuary covers an area of approximately 880 hectares, approximately eight square kilometers. Two rivers flow into the estuary (Knox and Kilner 1973), the Avon River from the northern tip while the Heathcote River enters from the southwest corner (Figure 2.1). The estuary has a long history of anthropogenic change and on the west part of the estuary is the Christchurch Wastewater Treatment Plant. In early 2009, the City Council started to construct an ocean pipeline to remove the treated waste water from the estuary. When this became operational in May 2010, estuarine conditions were expected to improve.



Figure 2.1 View of the Avon-Heathcote Estuary from the west, with the locations of the Avon and Heathcote Rivers, Waste Treatment Plant and the treated discharge point identified. From Swinscoe 2008.

From an ecological perspective, the estuary needs to be considered in conjunction with the adjacent oxidation ponds and the estuary's undeveloped margins. It is an excellent area to study the migratory and wetland bird species, invertebrates and salt marsh plant communities (Owen 1992).

Tides, river flow and salinity

The Avon-Heathcote Estuary has a micro-tidal range of approximately 2m, heavily influenced by prevailing winds and fluctuating river flow. Between Shag Rock and the end of the sandy spit lies the main outlet channel of the estuary. The average depth of the estuary is 1.4m. During spring tides, there is a microtidal range of 2m and 1.1m for neap tides (Marsden and Bressington 2009). During low tides approximately 80% of the estuary mudflats are exposed and there is about 56% tidal exchange per tide (Stephenson 1980). Both the Avon and Heathcote rivers are meandering, slow-flowing and spring fed rivers (Knox and Kilner 1973). Complete mixing of water column occurs when the flood tide spreads over the intertidal flats. According to Knox (1992), the salinity varies within the estuary on a daily and seasonal basis depending on the river discharge at any given time. It has been estimated that on each flood tide around $8.5 \times 10^6 \text{ m}^3$ of seawater enters the Avon-Heathcote Estuary.

Sediments

Sediments in the Avon-Heathcote Estuary come from the ocean, Avon and Heathcote Rivers (Knox and Kilner 1973) and consist of silt, clay, fine sands and shell fragments (Knox 1992). The river mouths are dominated by well-sorted sands at high-tide levels, and at low-tide levels close to the rivers the sediment consists of muddier silt (Knox and Kilner 1973). The mudflats around the mouth of the Heathcote are siltier than those of the Avon mouth because a third of the Heathcote catchment includes run-off from the fine erosion prone soils of the Port Hills (Owen 1992). At the mouth of the estuary the sediments consist of heavier and coarser particles. When particles coming down the river make contact with the salt water they stick together and fall down to the bottom where they form the fine

sediments that dominate the river mouths (Harris 1992). Annually about 80% of the incoming deposited material from the rivers collects in the estuary, river mouths and along the western shoreline (Knox 1992).

The main study species

Dominant estuarine macroinvertebrates include mussels, cockles, aquatic mudsnails, crustaceans and polychaete worms. These invertebrates are used worldwide as ecological indicator by scientists, environmental officers, managers and the public. They provide an early warning to scientists of ecological problems and are used to assess environmental change. According to Niemi and McDonald (2004), macroflora and macrofauna like aquatic macroinvertebrates, birds, fishes and vascular plants are useful ecological indicator species. This is because they are easy to identify and sample, they show quick responses to environmental changes, and because of the diversity of species, they represent different feeding habitats and life histories (Niemi and McDonald 2004).

In this research, *Amphibola crenata*, an endemic mudflat snail, was the chosen indicator species because it is one of the most conspicuous members of the estuarine benthos (Figure 2.2).



Figure 2.2 *A. crenata* and feeding (faecal) trails.

It is a mudsnail from the family Amphibolidae, a univalve, deposit feeding pulmonate marine snail (Phylum Mollusca, Class Gastropoda). This species was first collected by Cook on his voyage to New Zealand in 1769 (Watters 1964). It is endemic to, and characteristics of, most New Zealand intertidal estuaries (Jones and Marsden 2005).

This species is one of a few species of marine pulmonates in Australasia which lives between the marine and terrestrial environment (Watters 1964). It grazes on mud containing microorganisms on the surface of the tidal flats and is likely to be a good indicator of changes in nutrient conditions within estuarine ecosystems. According to Juniper (1982), this mudflat snail is a mobile deposit feeder, it ingest mainly inorganic surface sediments from the upper 15 cm, from which the microflora and fauna (bacteria, diatoms, flagellates etc. (Knox and Kilner 1973) and organic detritus (Jones and Marsden 2005) are assimilated as food. Knox and Kilner (1973) reported that the undigested waste is deposited as a faecal string behind each snail. Individual *A. crenata* are considered mature at around two years old when they reach approximately 20mm in shell length (Jones and Marsden 2005).

The first descriptions on the anatomy of *A. crenata* were provided by Quoy and Gaimard in 1832 (Watters 1964). It has been the subject of much research, early studies in 1927 by Thompson have been followed by detailed studies on the population dynamics and distribution, physiology, feeding, assimilation, reproduction, growth and the effects of environmental factors on its biology (Thompson 1929; Watters 1964; Jolly 1971; Briggs 1972; Voller 1973; Bennington 1979; Juniper 1982, 1987a, 1987b; Pilkington and Pilkington 1982, 1984a, 1984b; Shumway 1981, Shumway and Marsden 1982; Pechenik *et al.* 2003).

A. crenata is hermaphrodite and breeds from November to March in the South Island, August and May in the North Island (Briggs 1972). The mudflat snail protects its eggs within a gelatinous ring of mud (nidus). A total of 7,500 to 10,000 eggs can be found in each nidus which an adult lays every five days through the breeding season (Jones and Marsden 2005).

When the tide rises or during cold weather, mudflat mudsnails will stop grazing and burrow into the sediment. In the Avon-Heathcote Estuary feeding occurs during substrate exposure (Bennington 1979). The distribution of mudflat snails in the estuary is influenced by salinity (it is tolerant of a wide range of salinities), sediment (which affects burrowing, egg laying, and food), temperature and exposure by the tides. Bennington (1979) described the distribution of *A. crenata* as a) they are characteristics of soft mud/muddy sand in upper estuaries; b) they are common above the mid-tide level, with maximum densities occurring immediately below the mid-water level; c) juveniles prefer finer sediment than adults; and d) the distribution is limited by extreme pollution.

2.2 Objectives

The aims of the present study were to:

- 1) Quantify the densities and biomass of the mudsnail throughout the Avon-Heathcote Estuary; and
- 2) Relate the distribution and abundance of the mudsnail to physical and chemical variables such as salinity, percentage of total organic content and percentage of pore water of sediments.

2.3 Methods

Field work

A study on the distribution and abundance of *A. crenata* was carried out in the Avon-Heathcote Estuary (Figure 2.3) between November 2008 and July 2009. The Avon-Heathcote Estuary was divided into 182 sampling points using a grid created by staff in the Department of Geography. The coordinates for each point were generated using Geographical Position Systems (GPS). Before heading out to do the field sampling, the GPS coordinates of the 182 points were transferred into GPS Garmin 60CSx device. The sampling of *A. crenata* was done by random sampling at each of the points as shown in the map. For further descriptive purposes, the estuary was divided into seven zones which had similar environmental characteristics such as sediment types and distance from the edge of the estuary.

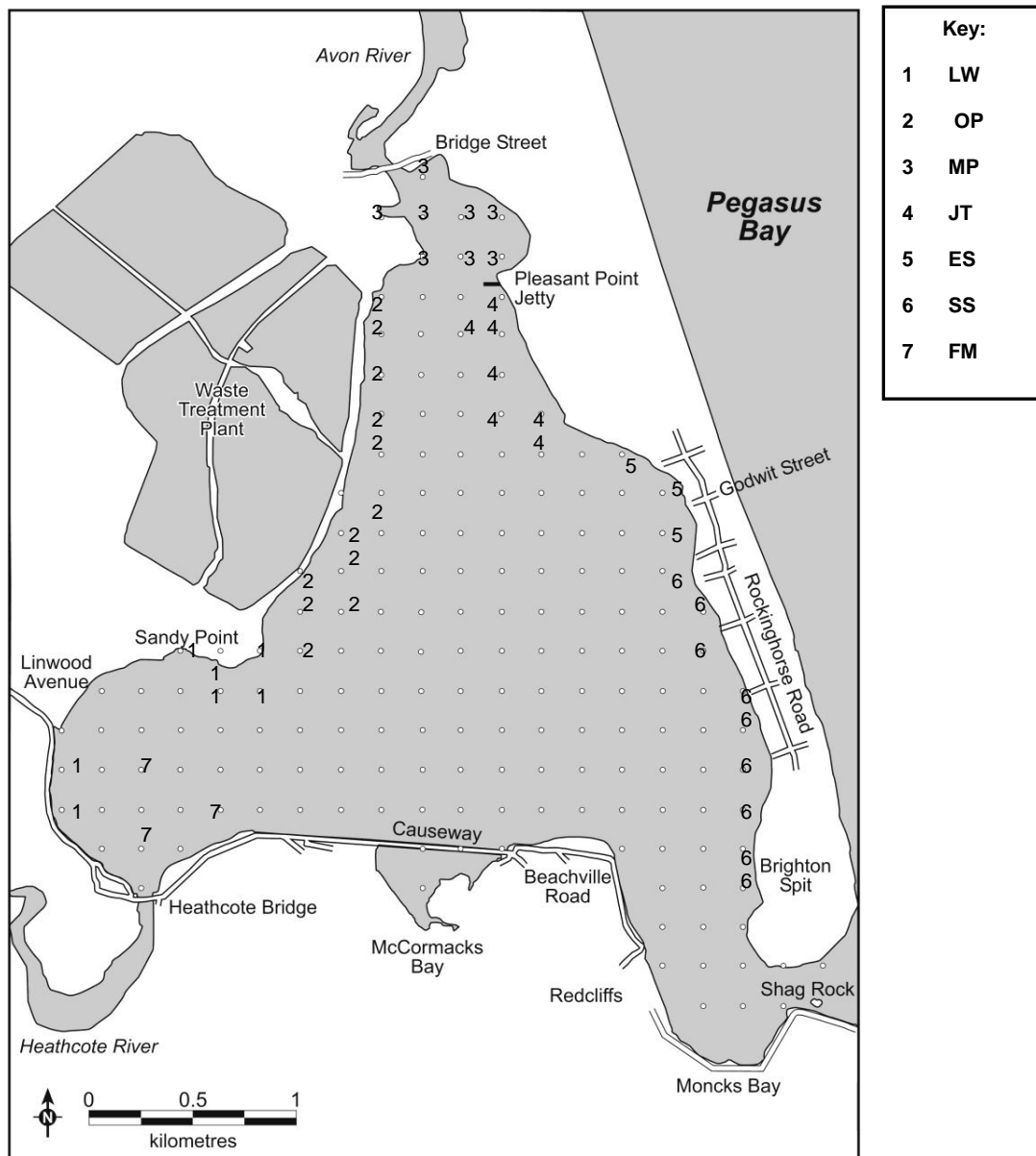


Figure 2.3 Map of the Avon-Heathcote Estuary showing the sampling locations used in the field survey. The estuary was divided into seven zones based on environmental characteristics. The zones are Linwood (LW); Oxidation Ponds (OP); Mt. Pleasant Yacht Club (MP); Pleasant Point Jetty (JT); Ebbtide St. (ES); Southshore (SS); and Ferrymead (FM).

***Amphibola crenata* sampling**

At each of the points, a random 0.5m X 0.5 m quadrat was placed in a homogenous area within 10m of the grid point. In each quadrat, all of the mudflat snails on the surface of sediment were collected. Then, within the quadrat, 5 cm depth of sediment was dug up and washed through a 1 mm mesh sieve. All of the mudsnails that had been collected were stored in plastic bags, labelled and returned to the laboratory. The percentage cover of the flora and fauna on the surface were recorded within the quadrats. For the subtidal sites sampling was done by boat between mid and low tide. An anchor-net dredge was used in this sampling program (Figure 2.4). The dimension of the anchor was 52cm in length and 33cm in width and 7.5 kg in weight. The net was 45cm in length and 49cm in width. The anchor-net dredge was lowered into the estuary once the particular site had been reached by referring to GPS coordinates. The mudsnails that were found were collected and placed in the plastic bag with labels. Other species that were found in the dredge were also recorded.



Figure 2.4 Anchor net dredge that was used benthic sampling using a boat.

In the laboratory, extraneous mud was washed from each snail with sea water and any algal growth on the shells removed using a brush. Each snail was weighed using an electronic scales brand Mettler PJ 300. The length (distance between the apex of the whorl and the lips (Briggs 1972) was measured (millimeter) using plastic vernier calipers (Figure 2.5). Pieces of tinfoil were cut to a size that was large enough to enclose the snail and these were numbered using a permanent marker and weighed. The mud snails in the tin foil were reweighed and placed on a tray which was dried in an oven at 65 °C for 72 hours (3 days). Samples were reweighed on the fourth and fifth day onwards to monitor any change of weight and to obtain the constant whole body dry weight.

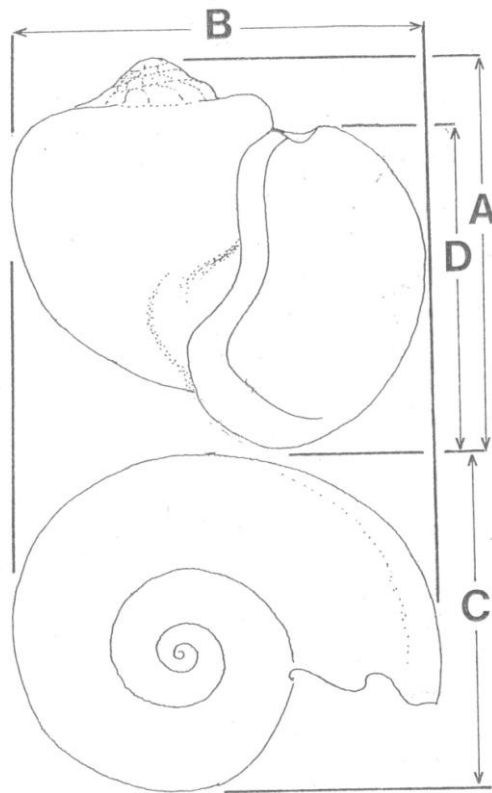


Figure 2.5 General shell dimensions of adult *Amphibola crenata*. A = shell length, B = maximum shell width, C = minimum shell width, and D = aperture length. From Bennington (1979).

Pore water and organic content of sediment

One sediment core was collected at each sampling site using a 50 mm diameter and 60 mm deep plastic pipe. A similar sediment core was also obtained from the sediment collected in the anchor net dredge. The sediments were stored in air tight plastic containers and kept in a temperature controlled room at 4° C. A sample from the core sample was weighed to obtain the wet weight of the sediment to the nearest 0.001g. The samples were then dried at 60 °C overnight. The sample was reweighed and the percentage of pore water (the amount of water that the sediment holds) was calculated as: $(\text{Wet weight} - \text{Dry weight}) / \text{Wet weight} \times (100)$. The dried sediment was then ashed at 425 °C for five hours. The ashed sediment was reweighed to the nearest 0.001g and the percentage of organic content in the sediment at each site was calculated as: $(\text{Dry weight} - \text{Ashed weight}) / \text{Dry weight} \times (100)$.

Water sampling

Sea water samples were obtained at low tide by digging a shallow hole in the sediment and collecting the surface interstitial water which had drained into the depression. For the boat samples, a water sample was collected at the each of the points where the boat had stopped. Water samples were put into 250 ml plastic containers and taken to the laboratory. They were retained in the laboratory until the muddy particles had settled and the salinity was measured using an Atago hand refractometer.

Data analyses

The abundance and biomass data were compared using analysis of variance using EXCEL. Quadrats or sites containing no *A. crenata* were excluded from the statistical analyses. The relationships between biomass, abundance and environmental variables were examined using Spearman rank correlation analysis, Poisson and Binomial models.

2.4 Results

2.4.1 *Amphibola crenata* densities

A. crenata was not equally distributed within the Avon-Heathcote Estuary (Figure 2.6) and individuals were distributed mainly within 400m from the estuary edge. However, no *A. crenata* were found in the southern part of the estuary, along the Causeway, McCormacks Bay, and Beachville Road. Also, there were no mudsnails in samples from the sandflats in the centre of the estuary. Close to the Linwood canal, the densities of *A. crenata* were 1-50 individuals/m². The highest densities of more than 151 individuals/m² were found near to Sandy Point and 66.7m from the Pleasant Point Jetty. At the Oxidation Ponds, Mt. Pleasant Yacht Club and Ebbtide Street, the densities ranged from 1-150 individuals/m². At Southshore and along Rockinghorse Road, densities ranged from 1-100 individuals/m² and at Ferrymead, 51-100 individuals/m² were found. There were similar densities 133m and 167m from the Heathcote Bridge while 1-50 individuals/m² were found about 400m from the bridge.

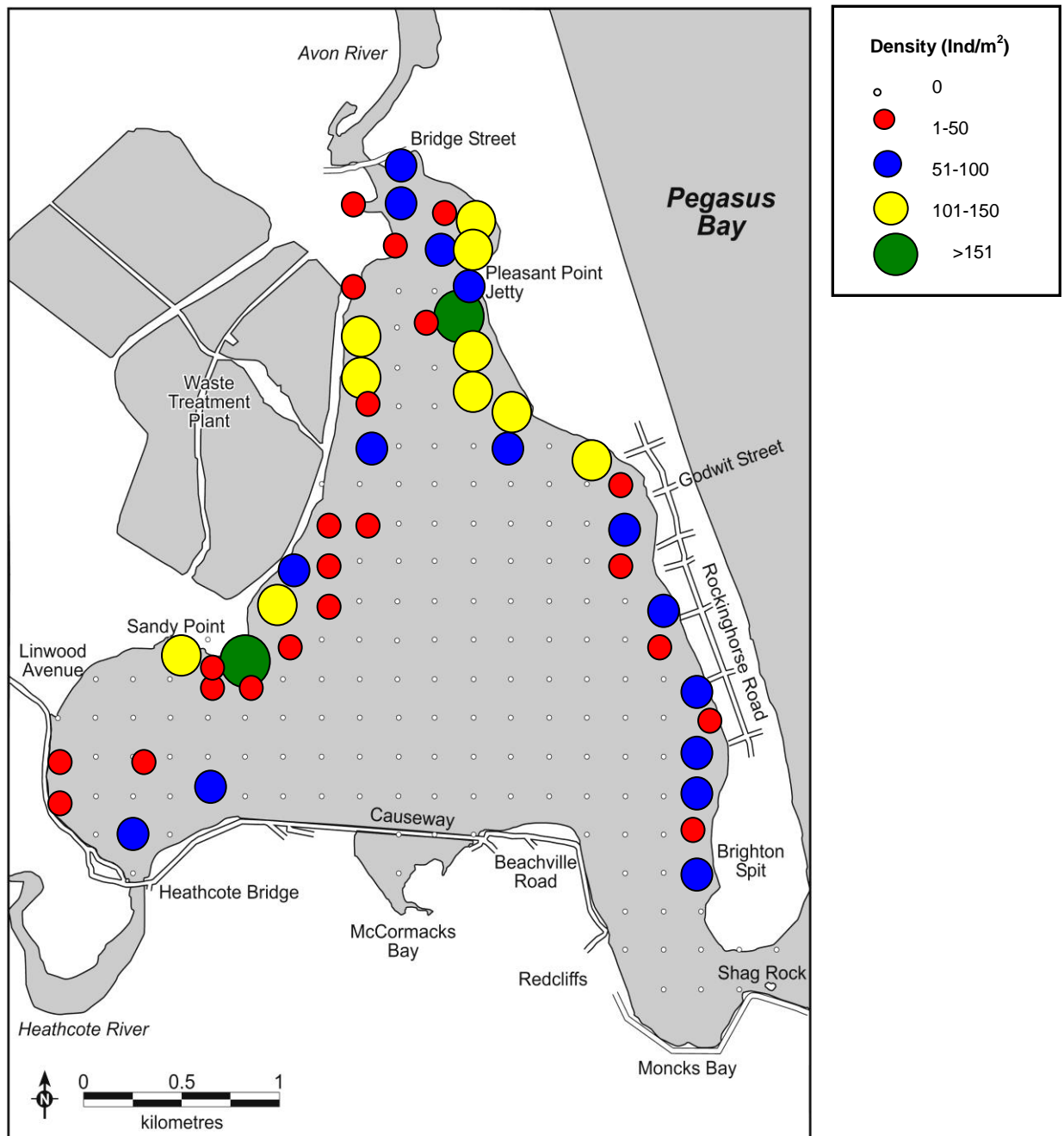


Figure 2.6 Density (individual/m²) of *A. crenata* at each sampling point in the Avon-Heathcote Estuary.

Densities of *A. crenata* were similar between site groups (one-way ANOVA $F= 0.524$, $P> 0.05$) (Table 2.1). The average densities ranged from 55 individuals/m² to 94 individuals/m² (Figure 2.7).

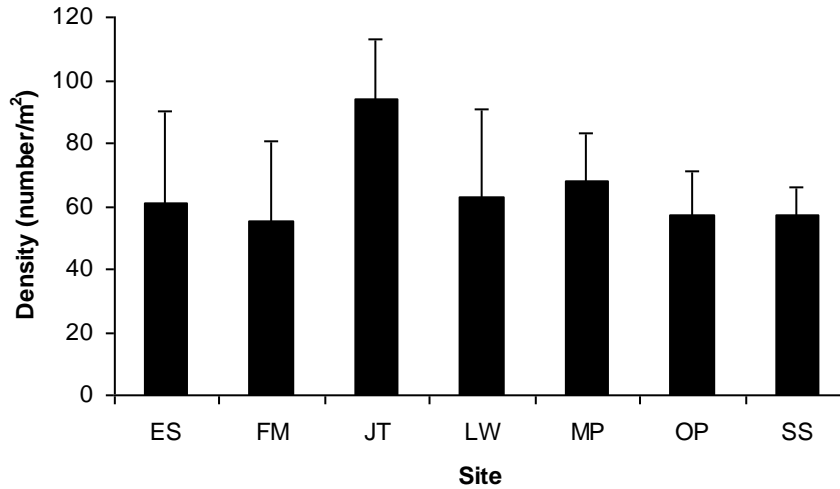


Figure 2.7 Density (number/m² \pm SE) of *A. crenata* at Ebbtide St. (ES); Ferrymead (FM); Pleasant Point Jetty (JT); Linwood (LW); Mt. Pleasant Yacht Club (MP); Oxidation Ponds (OP); and Southshore (SS).

Table 2.1 Results of analysis of variance comparing the density of *A. crenata* between sites.

Source of Variation	SS	Df	MS	<i>F</i>	<i>P-value</i>
Between sites	7573.982	6	1262.330	0.524	0.787
Within sites	101170.508	42	2408.822		

2.4.2 *Amphibola crenata* biomass

Biomass values were calculated only for sites where mudsnails were found. Biomass was variable and depended on location (Figure 2.8). Close to Linwood, the biomass ranged from 3.1 g/m² to more than 4.1 g/m² and near to Sandy Point the biomass ranged from 1.1 – 3.0 g/m². At the Oxidation Ponds, the biomass ranged from 1.1 – 4.0 g/m². Biomass was higher a 100m north and 300m south of the effluent discharge point with 3.1 – 4.0 g/m². At Mt. Pleasant Yacht Club, the biomass of mudsnails was 2.1 – 3.0 g/m², however, to the west of this yacht club the biomass was slightly higher with more than 4.1 g/m². At Pleasant Point Jetty, 100 meters from the edge of the estuary, the biomass was 2.1 – 3.0 g/m². Approximately 200 meters from the edge of the estuary the, the highest biomass was more than 4.1 g/m². At a distance of 266.7m from the edge of the estuary, the biomass was slightly lower with 0.1 – 1.0 g/m². At Ebbside Street, the biomass ranged from 1.1 – 4.0 g/m² and at Southshore, the biomass ranged from 1.1 – 3.0 g/m². At Ferrymead, the biomass was 1.1 – 2.0 g/m² some 133 meters from the edge of the estuary, 2.1 – 3.0 g/m² at 166.7 meters from the edge and 0.1 – 1.0 g/m² 400 meters from the estuary edge.

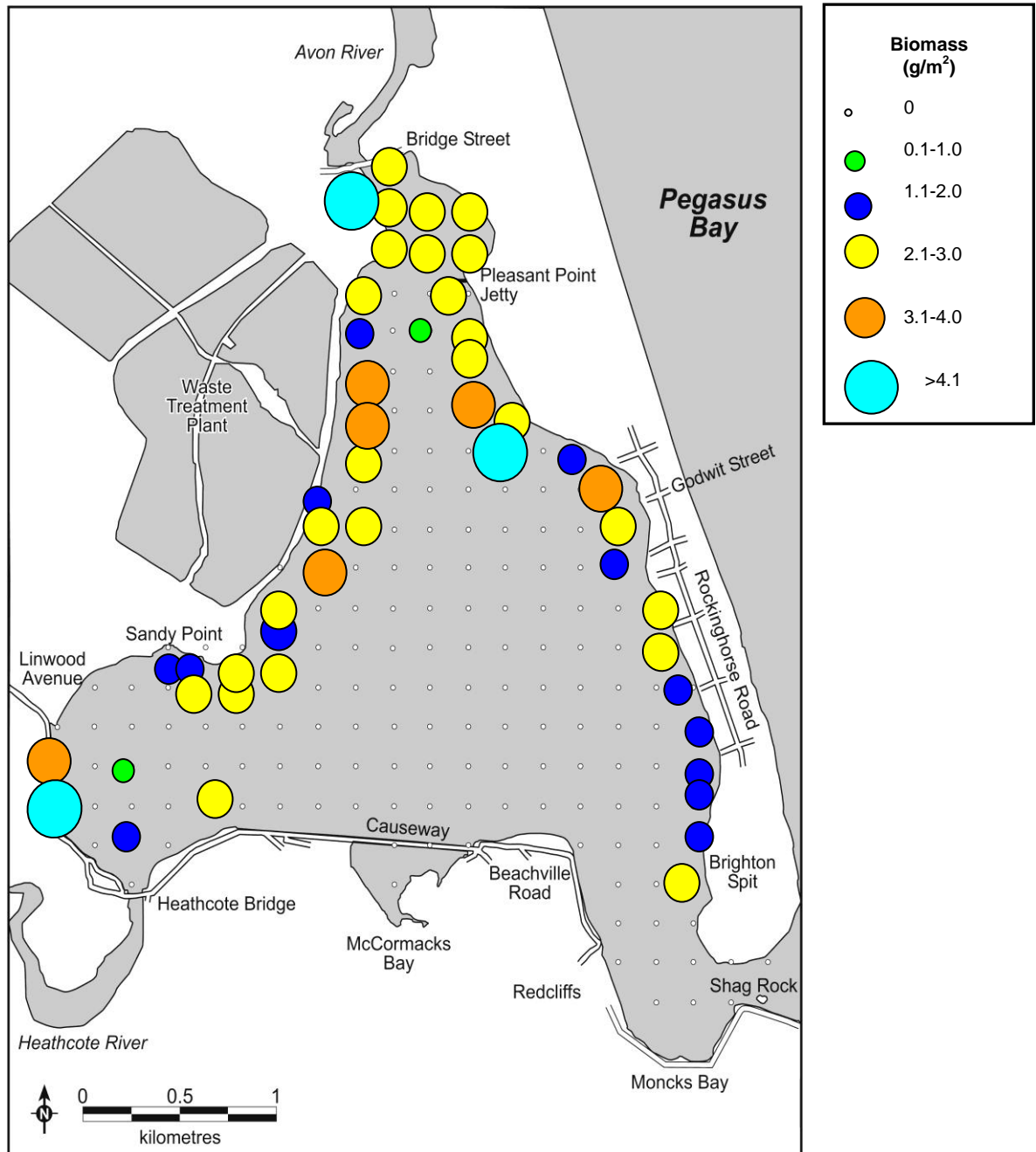


Figure 2.8 Biomass (g/m²) of *A. crenata* at each of the sampling point in the Avon-Heathcote Estuary.

The average biomass of *A. crenata* ranged from 1.7 g/m² to 2.8 g/m² (Figure 2.9). The biomass (g/m²) of *A. crenata* was similar and not varied between site groupings (one-way ANOVA $F= 1.434$, $P> 0.05$) (Table 2.2).

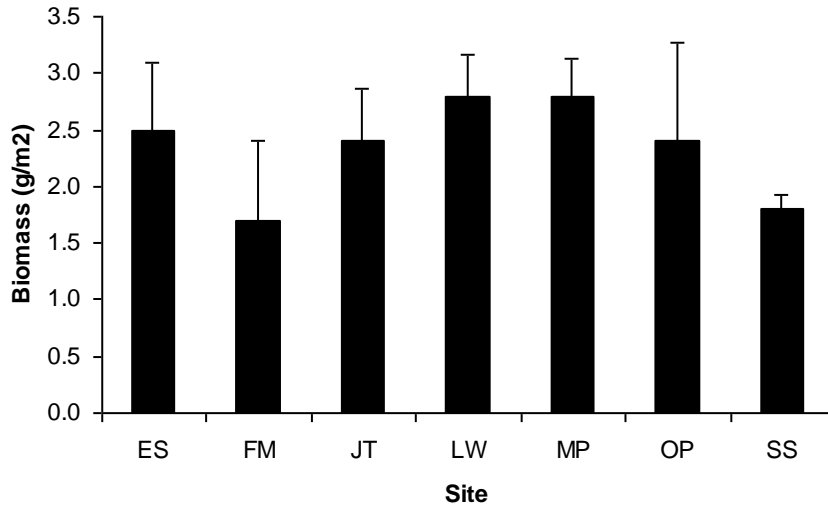


Figure 2.9 Biomass (g/m² \pm SE) of *A. crenata* at Ebbtide St. (ES); Ferrymead (FM); Pleasant Point Jetty (JT); Linwood (LW); Mt. Pleasant Yacht Club (MP); Oxidation Ponds (OP); and Southshore (SS).

Table 2.2 Results of analysis of variance comparing the biomass of *A. crenata* between sites.

Source of Variation	SS	df	MS	<i>F</i>	<i>P-value</i>
Between sites	6.892	6	1.149	1.434	0.225
Within sites	33.653	42	0.801		

2.4.3 Organic content in sediments

The organic content in the sediments ranged from 0.8% to 4.4% (Figure 2.10). Highest values were recorded from the Ferrymead sites. The percent organic content in the sediments was similar and did not vary amongst site groupings (one-way ANOVA $F= 2.033$, $P> 0.05$) (Table 2.3).

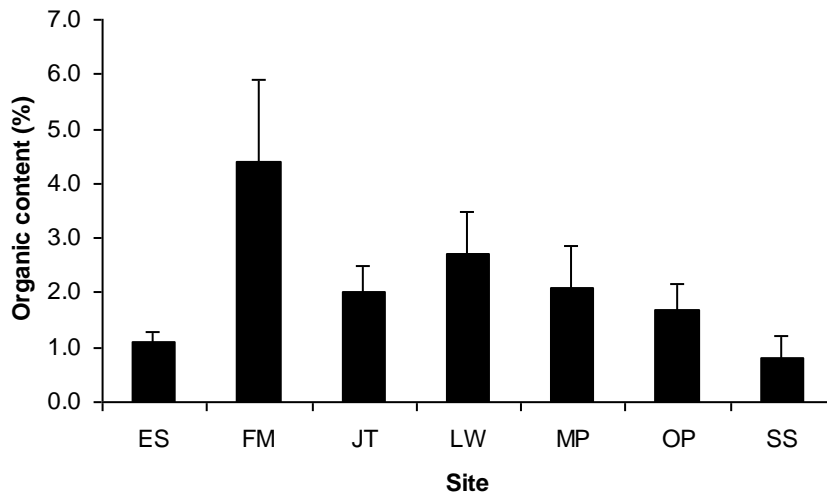


Figure 2.10 Percentage of organic content in sediments (\pm SE) at Ebbtide St. (ES); Ferrymead (FM); Pleasant Point Jetty (JT); Linwood (LW); Mt. Pleasant Yacht Club (MP); Oxidation Ponds (OP); and Southshore (SS).

Table 2.3 Results of analysis of variance comparing the percentage of organic content of sediments between sites.

Source of Variation	SS	df	MS	<i>F</i>	<i>P-value</i>
Between sites	20.470	6	3.412	2.033	0.104
Within sites	36.926	22	1.678		

2.4.4 Porosity of sediments

The pore water content of the sediment ranged from 22.2% to 30.5% (Figure 2.11) and was similar amongst site groupings (one-way ANOVA $F=0.771$, $P>0.05$) (Table 2.4).

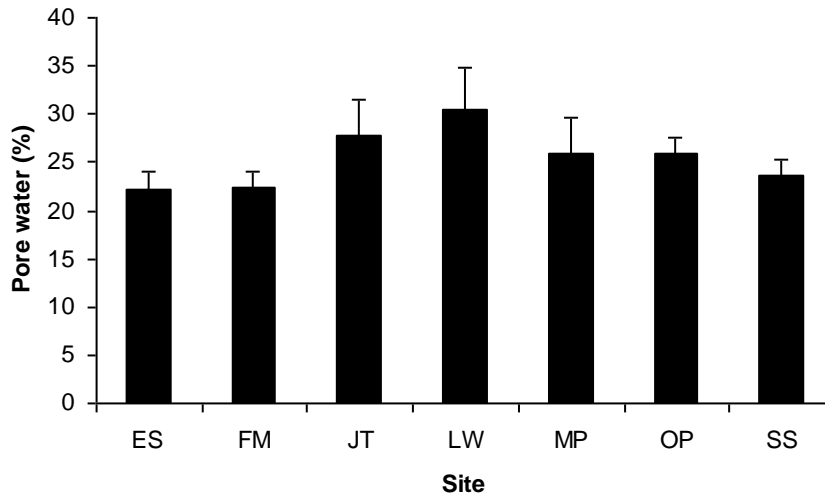


Figure 2.11 Percent porosity of sediments (\pm SE) at Ebbtide St. (ES); Ferrymead (FM); Pleasant Point Jetty (JT); Linwood (LW); Mt. Pleasant Yacht Club (MP); Oxidation Ponds (OP); and Southshore (SS).

Table 2.4 Results of analysis of variance comparing the percentage of porosity of sediments between sites.

Source of Variation	SS	df	MS	<i>F</i>	<i>P-value</i>
Between sites	166.369	6	27.728	0.771	0.600
Within sites	827.032	23	35.958		

2.4.5 Relationships between density, biomass and environmental variables

As expected, there were significant correlations between the physical and chemical variables of the sediment (Table 2.5). The percentage of organic content was strongly correlated with percentage of pore water ($r_s = 0.790$) (Table 2.5). From the analysis of the 182 points surveyed, there was a strong positive correlation between *A. crenata* density and biomass (Spearman $r_s = 0.678$). There was a negative correlation between the *A. crenata* density and the distance from the edge of the estuary ($r_s = -0.385$) and also negative correlation between *A. crenata* biomass and the distance from the edge of the estuary ($r_s = -0.460$). *A. crenata* densities and biomass were lower as the distance increased from the edge of the estuary.

The Spearman's rank correlation coefficient showed there was a negative correlation between salinity and density ($r_s = -0.323$) and biomass ($r_s = -0.423$) (Table 2.5). With increasing salinity, the density and biomass decreased. Similarly, the Poisson model suggested that, as the distance from the edge of the estuary and salinity increased, the density of *A. crenata* decreased (Table 2.6 and Table 2.7). The interaction term suggested that the rate of decrease in biomass with distance from the edge of the estuary was not consistent with all levels of salinity. The binomial model showed the same effect where *A. crenata* were present close to the shore and associated with low salinities (Table 2.8). All the models consistently indicate that mudsnails were distributed close to the estuary edge.

Table 2.5 Spearman rank correlation coefficients relating *A. crenata* density and abundance to environmental variables.

	<i>Points No.</i>	<i>Distance from edge (m)</i>	<i>Mudsnail density (ind/m²)</i>	<i>Biomass (g/m²)</i>	<i>Salinity</i>	<i>% Pore water</i>	<i>% Organic Content</i>
Points No.	1						
Distance from edge (m)	0.055	1					
Mudsnail density (ind/m ²)	0.000	-0.385	1				
Biomass (g/m ²)	-0.081	-0.460	0.678	1			
Salinity	0.315	0.277	-0.323	-0.423	1		
% Pore water	-0.286	0.123	-0.038	0.048	-0.105	1	
% Organic content	-0.311	0.068	0.002	0.071	-0.094	0.790	1

Table 2.6 Poisson model relating the density of *A. crenata* with the distance from the edge of the estuary and salinity.

	Estimate	Standard error	P-values
Intercept	5.269	0.387	<0.001
Distance	-0.007	0.001	<0.001
Salinity	-0.047	0.015	<0.001

Density = Distance + Salinity + Distance

Table 2.7 Correlation coefficients relating *A. crenata* biomass with the distance from the edge of the estuary and salinity.

	Estimate	Standard error	P-values
Intercept	0.396	0.439	<0.001
Distance	-0.007	0.002	<0.001
Salinity	-0.093	0.015	<0.001
Distance: Salinity	<0.001	<0.001	<0.001

Biomass = Distance + Salinity + Distance* Salinity

Table 2.8 Binomial model testing the absence and presence of *A. crenata* with distance from the edge of the estuary and salinity.

	Estimate	Standard error	P-values
Intercept	4.432	1.037	<0.001
Distance	-0.010	0.002	<0.001
Salinity	-0.122	0.033	<0.001

Density = Distance + Salinity, family= binomial

2.5 Discussion

***Amphibola crenata* densities and biomass in the Avon-Heathcote Estuary**

As in other estuaries around New Zealand, *A. crenata* was found to be an important component of the macrofauna in the Avon-Heathcote Estuary. Mudsnaills were found on the eastern and western shoreline of the estuary. This supports research by Bennington (1979) who found that this species was common above mid-tide level, with maximum densities occurring immediately below mid-water level. In the present study *A. crenata* was found in higher abundances in the lower (mouth) regions of the Avon compared to the Heathcote River. This contrasted with the research in the 1950s and 1960s, when mudsnails were abundant at the river mouths of both the Avon and Heathcote rivers and near to the Brighton Spit (Bruce 1953; Clark 1957; Williams 1960; Kilner 1969). In the present study, populations of *A. crenata* close to the Avon River included larger individuals of more than 10 mm shell length, densities up to 150/m² and biomass between 2.1 – 3.0 g/m². This finding was similar to studies in 1992, when individuals of more than 10mm shell length individuals dominated the eastern margin of the estuary at the Avon River with densities up to 200/m² (Marsden and Knox 2008).

The largest individuals of *A. crenata* were found close to Linwood Avenue near to Sandy Point where adult *A. crenata* ranged between 22.0 – 25.0mm in shell length with an estimated biomass of 2.1 – 3.0 g/m². This area consisted of firm mud or sand (Robertson *et al.* 2002) and it is known that adults of *A. crenata* prefer such sediment. Small *A. crenata* are known to favour finer sediments (Bennington 1979). Although this site recorded the largest mudsnails, the seaweed *Gracillaria spp.* dominated the mudflats from 400 meters from the edge of the estuary and beyond and no mud snails were found. At Pleasant Point Jetty the average density was also high with more than 151/m² with an estimated biomass of 2.1 – 3.0 g/m². Since 1979 adults of *A. crenata* have dominated the eastern shoreline of the Avon-Heathcote Estuary and Pleasant Point Jetty (Bennington 1979).

The findings from the present survey suggest that *A. crenata* did not occur at lower-tidal levels and there was a strong negative correlation between the density and biomass of *A. crenata* with the distance from the estuary edge. As the distance increased towards the centre of the estuary, the density and biomass of *A. crenata* decreased. In 1969, the *A. crenata* distributions extended further into the central mudflats of the estuary (Marsden and Knox 2008). In the present study the mudsnail population was reduced at lower tidal levels. The rivers and water channels that flow from the Avon River and Heathcote River might be responsible for restricting mud snails from the centre of estuary. Here the sediments were softer and muddier toward the river channels and sampling was carried out using a boat at such locations. The different technique used to collect the mudsnails by boating may have influenced the results and cockles were regularly found in the sediment. This research confirms that individuals of *A. crenata* prefer the middle zone of environmental condition particularly those which affect exposure and submergence such as height above datum, distance from channel and distance from shore (Rodrigo 1985).

Environmental interactions with *Amphibola crenata*

In the present study, there was a significant relationships between mudsnail density, biomass and salinity. As the salinity increased, the mudsnail densities and biomass decreased significantly. Bennington (1979) also found that the distribution of mudsnails in the estuary was influenced by salinity (it is tolerant of a wide range of salinities).

There was no significant difference in the porosity of the sediments between sites. It was expected that the porosity might differ amongst sites because of differences in sediment particle size. Detailed substrate analysis was not undertaken, however, a generalized description of the substratum of the estuary is given by Robertson *et al.* (2002). Pore water or porosity of sediments describes the spaces between the grains of the material like rock, sand and soil or so called pores. For example clays can hold a large volume of water per volume of bulk material, however they do not release water rapidly. Clays which typically have

very low hydraulic conductivity also have very high porosities. There was also no correlation between the porosity in sediments and density of *A. crenata*.

There was no significant relationship between the total organic content of sediment and *A. crenata* density. This finding contrasts with that of Watters (1964) who found that the density and size of *A. crenata* was associated with organic content and a high silt-clay content. The size of mudsnails in the present study increased at lower-tidal levels and this result has also been found for other New Zealand estuaries, Hoopers Inlet, Otago (Watters 1964); Whangateau Harbour, Northland (Briggs 1972) and including the Avon-Heathcote Estuary (Clark 1957; Kilner 1969; Knox and Kilner 1973; Voller 1973; Bennington 1979).

2.6 Conclusions

The *Amphibola crenata* population was highly variable within the Avon-Heathcote Estuary and the density and biomass correlated with the distance from the estuary edge and salinity. This distribution is most likely a direct consequence of habitat selection by juvenile and adult mudsnails. The present study was planned in association with another recent study which measured the water depth and sediment composition of the estuary, using the same grid references used in the present study. These results are not yet available and so it was not possible to construct a more detailed mathematical model for mud snail densities and abundances. However, there were some clear patterns in the *A. crenata* abundances. The values recorded as part of this study will form an excellent baseline to evaluate potential changes that might occur as a consequence of changes in the nutrient conditions, now that treated sewage waste has been removed from the Avon-Heathcote Estuary.

Chapter 3: SEASONAL STUDY

3.1 Introduction

A wide diversity of fauna is found on intertidal estuarine flats and organisms have the ability to cope with variable environmental conditions (McClusky 1981). The composition of soft sediments (Thrush 1991) and the steep and variable gradients (Kilner 1969) also provide a suitable habitat for marine benthic organisms. The structure of benthic communities is determined by anthropogenic influences (Malloy *et al.* 2007) and also environmental gradients (Edgar and Barret 2002). Other factors that may affect the spatial patterns of estuarine species are biotic and abiotic factors, such as food sources, sediment characteristics, predator behaviour, competition and veliger settlement (Thrush 1989). Environmental conditions, as well as interaction with other species, also affect community structure (Dayton 1971). These can affect the distribution of species that occur over a wide range of habitats or species that are confined in a narrow habitat (Chainho *et al.* 2006). Organisms such as gastropod molluscs that inhabit the high intertidal zone are exposed to high environmental stress and their abilities to maintain basic metabolic requirements is a key element in their survival in the marine ecosystem (Shumway 1982).

Deposit feeders and benthic microorganisms are influenced by seasonal factors such as temperature, light and nutrient availability hence the mutual interaction between them can be affected by seasonal variation (Juniper 1987a). *Amphibola crenata* is subjected to many biotic and physical variables within estuaries and its distribution may be limited by salinity, exposure time, substrate composition and season (Bennington 1979). The distribution of *A. crenata* varied between seasons in the Hoopers Inlet, seaward side of the Otago Peninsula, New Zealand (Watters 1964). More individuals were found on the mudflat during spring and summer compared to winter. During winter fewer individuals were active on the mud surface on cold days and also when there was an increase in fresh water contributed by rain and snow. In addition, adults tolerated a lower range of salinity and also a higher temperature compared to juveniles (Watters

1964). There was also an interaction between mudsnail density and seasonal effects in the regulation of bacterial numbers (Juniper 1987a).

Studies by Britton (1985) showed that the recruitment period of hydrobiid snails such as *Hydrobia acuta* took place in May and June which was the beginning of summer in the southern lagoon of Tunis, Tunisia. The most favorable condition for growth occurred in autumn, when the salinity dropped due to increased rainfall. *Iravadia sakaguchii*, a gastropod belonging to family Iravadiidae, found in brackish water of Japan also showed a seasonal distribution (Kobayashi and Wada 2004). This species increased between the spring and summer (April, June and August 2001) and declined between autumn and winter (October and January 2002) in the intertidal mudflat in the Waka River Estuary, central Japan. The reproductive season of gastropods varies considerably as follows: spring for *Assiminea japonica* Martens, 1877 (Kurata and Kikuchi 2000), summer for *Hydrobia ventrosa* Montagu, 1803 (Barnes 1990), *Angustassiminea castanea* Westerland, 1883 (Kurata and Kikuchi 2000) and late winter-spring and summer-autumn for *Hydrobia ulvae* Pennant, 1777 (Anderson 1971; Fish and Fish 1974; Barnes 1990; Hauboies *et al.* 2002).

The distribution and abundance of deposit feeding gastropods is associated with sediment composition. Forbes and Lopez (1990) found that the deposit-feeding gastropod *Hydrobia truncate* population density and body size was greater in muddy compared to sandy habitats. Optimum growth was recorded in fine sediments rather than coarse-grained sediments. Previous studies have shown a positive correlation between body size and population density of hydrobiid gastropods with silt-clay content of sediment (Newell 1965; Fish and Fish 1974). Sediment particle size limits the food availability of organic detrital matter and living microbes to deposit feeders (Lopez and Levinton 1978). *Terebralia palustris* (Potamididae: Gastropoda) preferred to inhabit muddy rather than sandy substrates (Rambabu *et al.* 1987). Changes in sediment composition may also influence the colonization of benthic species (Chainho *et al.* 2006).

The ecological health of an ecosystem can be assessed through short term or long term monitoring programs (Brady and Francis 2010). Species abundance

and fluctuations can be detected by measuring changes in physical or chemical conditions or by following the distribution or abundance of organisms.

In 2009, the Christchurch City Council established an ocean pipeline to remove treated wastewater from the Avon-Heathcote Estuary. The three kilometer underground pipe transports the wastewater from the oxidation ponds in the Christchurch Wastewater Treatment Plant into Pegasus Bay instead of releasing it into the Avon-Heathcote Estuary (Christchurch City Council 2010). This human induced action is expected to improve the estuarine conditions.

This study is a ‘pre-diversion’ investigation of a key benthic invertebrate, and contributes to a long-term and large scale assessment of the remediation of the estuary. Diversion of wastewater into the ocean is expected to lead to the loss of major source of organic matter for primary production (Walrond 2007). The reduction in nutrient conditions in the Avon-Heathcote Estuary could also change the invertebrate species composition of the mudflats. It could provide more available habitat for cockles and suspension feeding invertebrates. Species like mudsnails and polychaete worms may not favour such conditions as there would be less benthic algae and organic deposits in the mud (Miller *et al.* 2004). It is expected that *A. crenata* density will vary between sites with different nutrient conditions and that there will be seasonal differences in the population.

3.2 Objectives

The aims of the present study were to:

- 1) Assess seasonal changes in distribution, density and biomass of *A. crenata*; and
- 2) Investigate the relationships between the *A. crenata* population with physical and chemical variables such as tidal levels, percentage of total organic content and percentage of pore water.

3.3 Methods

Site Descriptions and Locations

Five sites were selected based on differences in salinity, sediment characteristics, fresh water input, nutrient inputs and macrofaunal community type (Table 3.1). There were a) Avon; b) Ferrymead; c) Pleasant Point Jetty; d) Oxidation Ponds; and e) Tern Street (Figure 3.1, 3.2 a-d). The Pleasant Point Jetty site was also used by National Institute of Water and Atmospheric research's (NIWA). The winter sampling was carried out from 7th July 2009 to 12th July 2009 while summer sampling was from 3rd December 2009 to 7th December 2009.

Table 3.1 Summary of main characteristics of sampling sites, modified from Marsden and Knox (2008).

Site	Sediment type	Current speed	Fresh water influence	Nutrient inputs	Macrofaunal community type
Avon (Avon river mouth)	Very soft mud / sand	Low	Low	Close (river)	River mouth
Ferrymead (Heathcote river mouth)	Fine silts/clay	Very low	Low	Close (river)	River mouth
Pleasant Point Jetty	Fine mud / sand	Low	High	Close (river)	Estuarine
Oxidation Ponds (200m south of main outfall)	Firm shell / sand and firm mud / sand	Low	Moderate	Close (CWTP)	Estuarine
Tern Street	Firm mud / sand	Moderate	Low - moderate	Close (river)	Marine / seagrass meadows



Figure 3.1 Satellite image of the Avon-Heathcote Estuary showing five sampling sites in the estuary. The GPS coordinates for each site are included in the Appendix 1. Source Google Earth 2010.



Figure 3.2 Images of sampling sites taken in June and July 2009; a) Avon; b) Ferrymead; c) Pleasant Point Jetty; d) Oxidation Ponds; and e) Tern Street.

Sampling regime

Sampling was carried out in 30 X 15 m area which is half the size suggested by Robertson *et al.* (2002). To avoid trampling at the NIWA sampling station, an area was created by running a measuring tape parallel to the water line 2.5 m down shore. At each of the sites, a 30 X 15 m plot was marked by placing a wooden stake at each corner with the aid of measuring tape. GPS coordinates for each corner were recorded to enable subsequent repeat surveys. A transect was established at each water level defined as high (the highest level reached by a body of water), mid (between high-tide and low-tide) and low (the lowest level reached by a body of water) levels. This plot was divided into 15 X 7.5 m and marked with temporary stakes.

***Amphibola crenata* sampling**

In each plot, five quadrats of 50 X 50 cm were randomly placed. All individuals of *A. crenata* within the quadrat were removed, counted and placed in marked plastic bags. To collect small individuals or juvenile *A. crenata*, the quadrats were dug to a depth of 5 cm and sieved with a 1 mm mesh. In the laboratory, extraneous mud was washed from each snail using seawater and the shell length (distance between the apex of the whorl and the lips (Figure 2.5) was measured using electronic calipers.

***Amphibola crenata* biomass analysis**

Twenty five mudsnails were chosen from each tidal level for the biomass analysis. Each mudsnails was placed in a piece of pre-weighed tin foil, large enough to enclose a single snail, and weighed using an electronic balance (Mettler PJ 300). The sample's number was marked on the tin foils using a permanent marker. All the samples were dried in an oven in a tray at 65°C for 72 hours (3 days). The samples were reweighed on the fourth and fifth day to obtain a consistent dry weight.

Collection of sediment samples

From each transect, three subplots were randomly chosen at each site and three samples of sediments were collected using a 50mm diameter and 60mm deep plastic pipe. Sediments were stored in 250 ml airtight plastic containers and kept in a temperature controlled room at 4° C for further analysis. The sediment samples were analysed to determine organic content of the sediment and pore water as previously described in Chapter 2.

Collection of water samples

Samples of water were obtained by digging a shallow hole in the sediment. The mixture of surface interstitial water that drained into the depression was collected into a 250 ml plastic container and taken to the laboratory. The samples then were retained in the laboratory until the muddy particles settled down and the salinity was measured using the Atago hand refractometer.

Data analyses

Densities and distribution data from the July and December 2009 sampling periods were analysed and compared. Descriptive statistics (frequency distribution and ANOVA) were calculated using EXCEL 2003 and PASW Statistics 18.0 software. These are discussed in the chapter where appropriate.

3.4 Results

3.4.1 *Amphibola crenata* densities

A. crenata density varied amongst sites and tidal level in winter and summer. In winter, densities varied between sites (two-way ANOVA $F= 32.5$, $P< 0.001$) and tidal levels (two-way ANOVA $F= 69.5$, $P< 0.001$) (Table 3.2). The Oxidation Pond site had a significantly higher number with a mean of 28 individuals/m² and there were similar densities, ranging from 7 individuals/m² to 11 individuals/m², at other sites. At the high-tide level, the mean of 24 individuals/m² was significantly higher than the low-tide level (5 individuals/m²). (Figure 3.3). There was a significant interaction between sites and tidal levels on the densities of *A. crenata* in winter (two-way ANOVA $F= 9.3$, $P< 0.001$) (Table 3.2).

In summer, *A. crenata* density varied amongst sites (two-way ANOVA, $F= 55.8$, $P< 0.05$) and with tidal level (two-way ANOVA, $F= 9.2$, $P< 0.05$) (Table 3.2). The oxidation ponds site had a significantly higher number with a mean of 60 individuals/m² compared to Avon with 6 individuals/m². Densities were highest at high-tide and mid-tide level with 30 individuals/m² compared to the low-tide level with 19 individuals/m² (Figure 3.3). There was a significant interaction between site and tidal level on the densities of *A. crenata* (two-way ANOVA $F= 6.8$, $P< 0.001$) (Table 3.2).

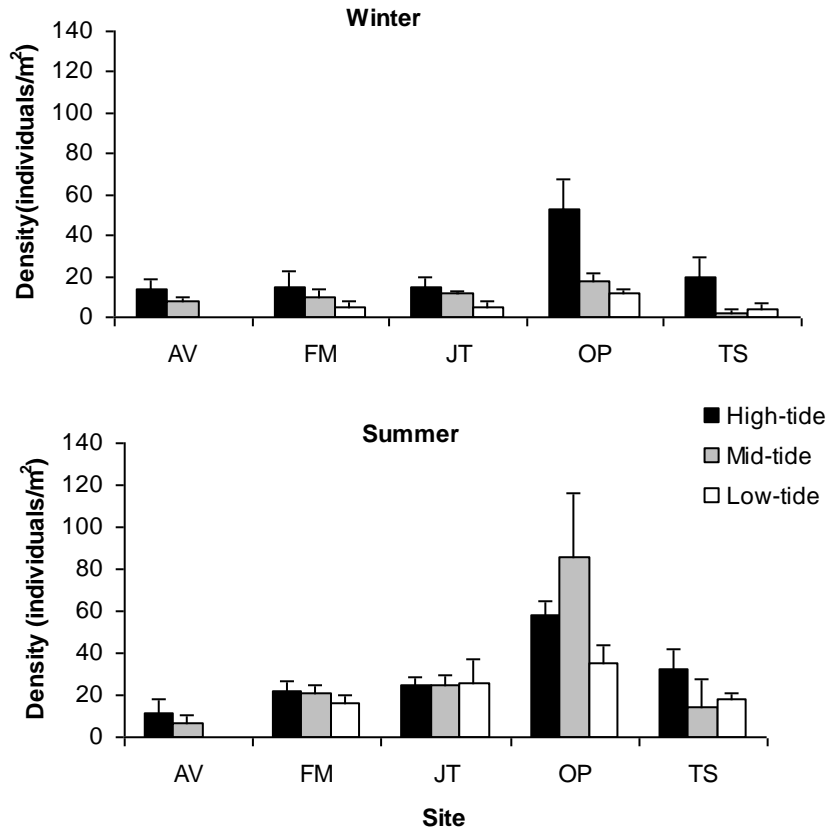


Figure 3.3 Seasonal densities (individuals/m² ± SE) between tidal levels at Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Table 3.2 Results of analysis of variance for comparing the density of *A. crenata* between tidal levels and sites in winter and summer.

Seasons	Source of Variation	SS	df	MS	F	P-value
Winter	Sites	4218.213	4	1054.553	32.548	0.001
	Tidal levels	4500.587	2	2250.293	69.453	0.001
	Interaction	2404.347	8	300.543	9.276	0.001
Summer	Sites	23891.733	4	5972.933	55.787	0.001
	Tidal levels	1965.787	2	982.893	9.180	0.001
	Interaction	5801.147	8	725.143	6.773	0.001

3.4.2 Seasonal variation in *Amphibola crenata* population structure

a) Population structure at the Avon River site

The mudsnail population at the Avon River site was dominated by larger individuals (21-25 mm) which formed 64.8% of the population at high-tide level and 65.1% at mid-tide level during winter. However, during summer, mudsnails of shell length 26-30 mm dominated the high-tide level (34.5%) and smaller mudsnails (11-15 mm) dominated mid-tide level (38.9%). Juvenile *A. crenata* (<5 mm) were found at the high-tide level (7.3%) during summer but none were found during winter. At the mid-tide level no juveniles occurred during summer or winter (Figure 3.4).

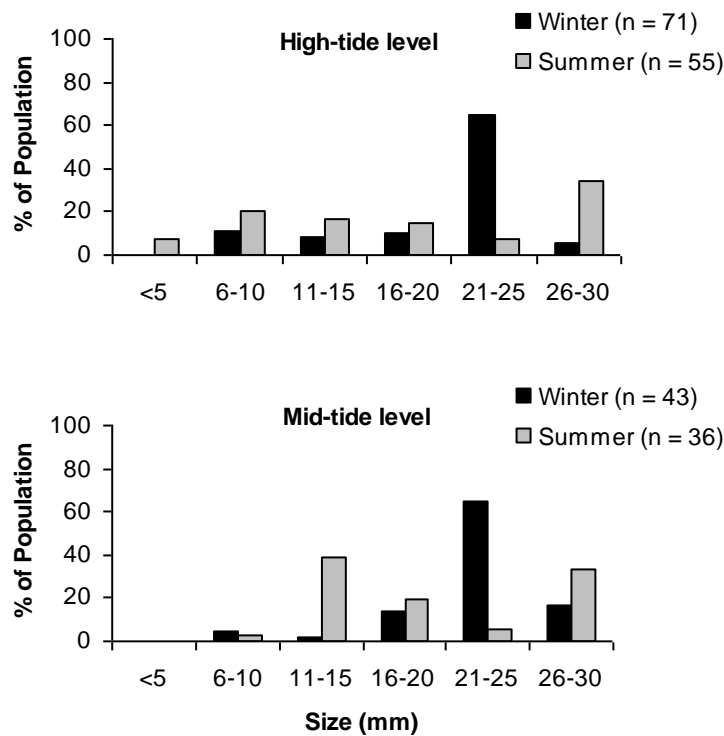


Figure 3.4 Avon River. Seasonal population structure of *A. crenata* at the high-tide and mid-tide levels.

Avon River frequency distribution

The numbers of mudsnails, grouped into length groups increased for all sizes groups from winter to summer at both tidal levels. The highest densities for the medium size class was recorded in summer (22 individuals/m²) compared to 3 individuals/m² at mid-tide level. Juvenile (<7.9 mm) were more abundant at the high-tide level in summer compared to winter when 12 individuals/m² were recorded. Adults (>18 mm) tended to decrease towards lower-tidal heights. No juveniles (<7.9 mm) were found at the mid-tide level during summer (Figure 3.5).

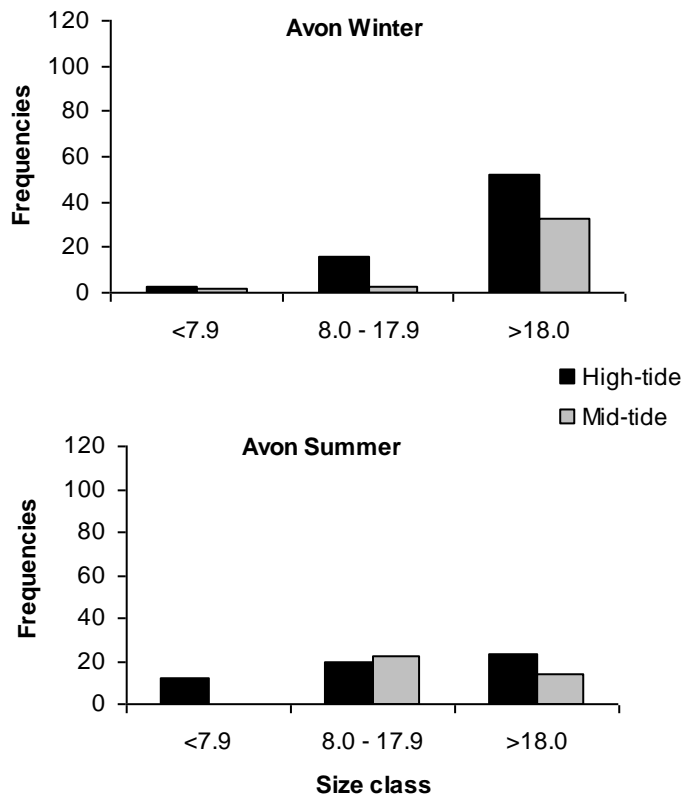


Figure 3.5 Number of individuals/m² within each size class at high-tide and mid-tide level at the Avon site. The size classes are the same as those used by Griffin and Thompson (1992).

b) Population structure at the Ferrymead site

The mudsnail population at Ferrymead was dominated by larger individuals (21-25 mm) which formed 72.7% of the population at the high-tide level, 70.3% at the mid-tide level and 95.7% at the low-tide level during winter. During summer, mudsnails of shell length 21-25 mm dominated the high-tide level (64.0%), mid-tide level (59.2%) and the low-tide level (46.9%). No juvenile *A. crenata* (<5 mm) were found at the mid-tide level during winter and none of this size range were found at low-tide level in summer. Mudsnails of shell length 11-15 mm were found at the low-tide level during winter and none of this size range were found at low-tide level in summer. Mudsnails of shell length 11-15 mm were found at the low-tide level (1.2%) during summer (Figure 3.6).

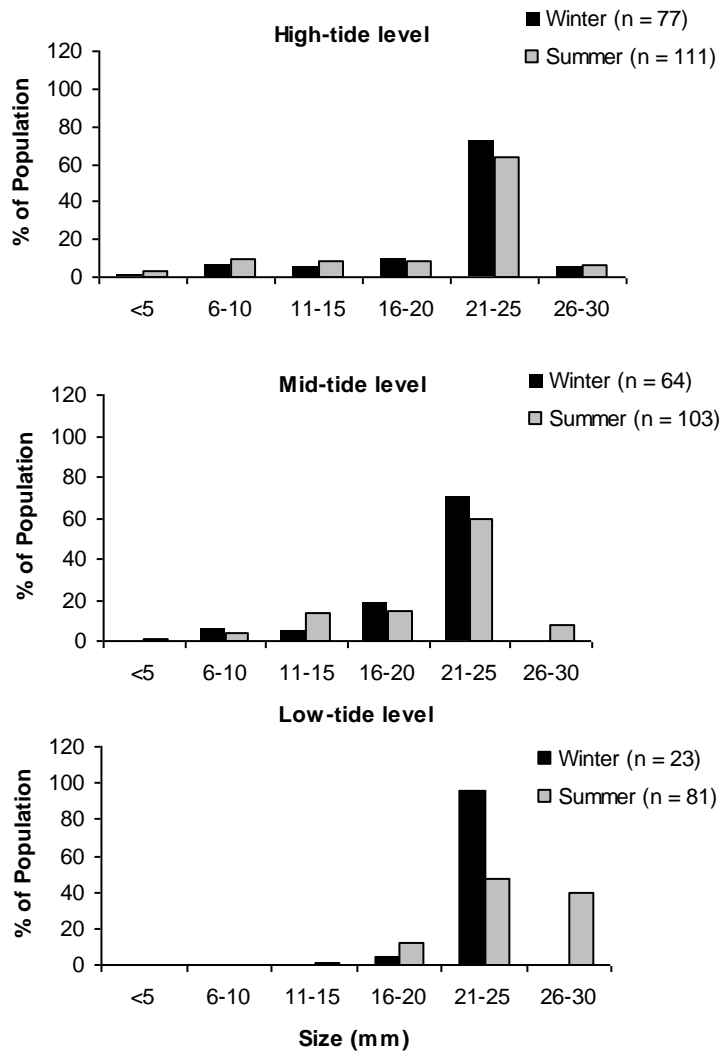


Figure 3.6 Ferrymead. Seasonal population structure of *A. crenata* at all tidal levels.

Ferrymead frequency distribution

The numbers of mudsnails, grouped into length groups increased for all size groups from winter to summer at all tidal levels. In Ferrymead, adults (>18 mm) mudsnails were found at all tidal levels in both seasons. Also, juveniles (<7.9 mm) were found at the low-tide level in both seasons. Medium sized mudsnails (8.0-17.9 mm) were found at the low-tide level which was 7 individuals/m² in summer but none in winter (Figure 3.7).

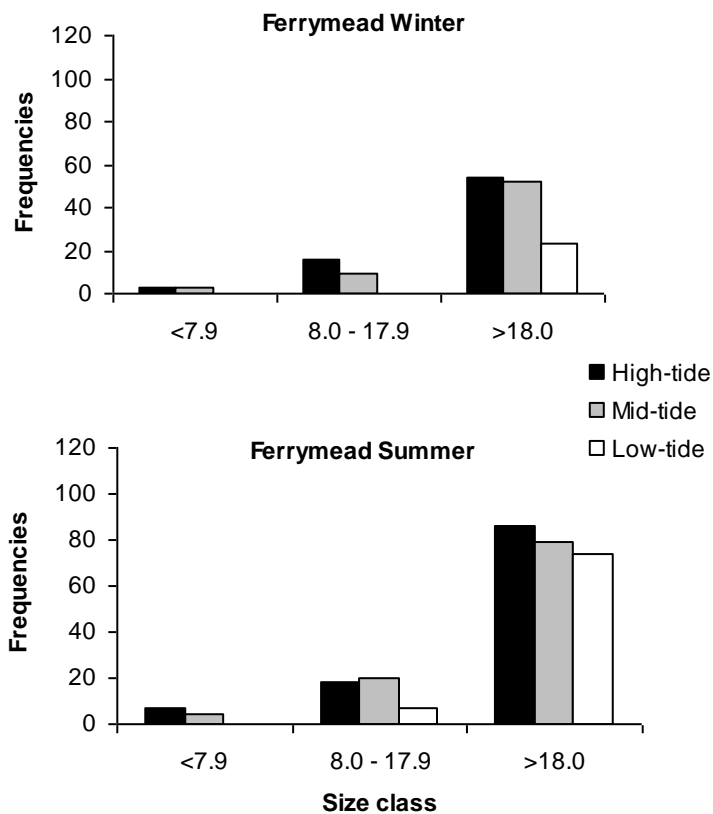


Figure 3.7 Number of individuals/m² within each size class at all tidal levels at the Ferrymead site. Sizes classes as in Griffin and Thomson (1992).

c) Population structure at the Pleasant Point Jetty site

The mudsnail population at Pleasant Point Jetty was dominated by larger individuals (21-25 mm) which formed more than 60% of the population at all tidal levels in both seasons. No juveniles (<5 mm) were found at the high-tide level in both seasons, however, they were found at the mid-tide (2.4%) and the low-tide (2.3%) level in summer. During summer, mudsnails of shell length 26-30 mm were found the high-tide level (1.6%) and at the mid-tide level (0.8%). However, no mudsnails from this size class were found at the low-tide level during winter or summer (Figure 3.8).

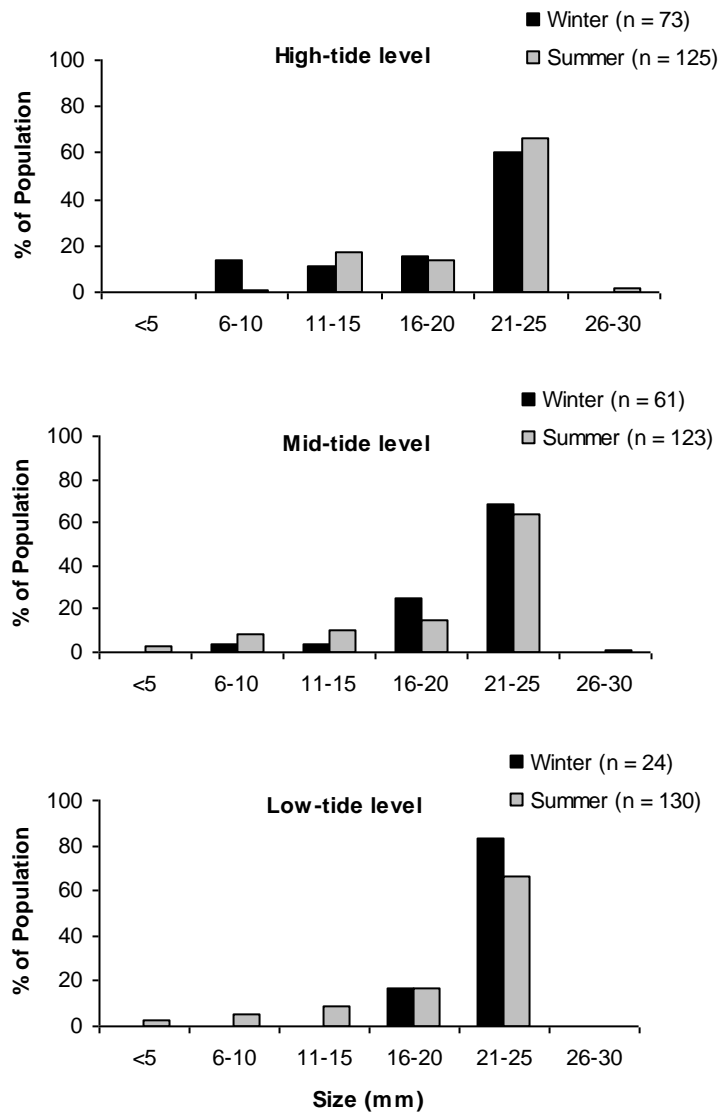


Figure 3.8 Pleasant Point Jetty. Seasonal population structure of *A. crenata* at all tidal levels.

Pleasant Point Jetty frequency distribution

Adult *A. crenata* (>18.0 mm) were found in high densities at all levels and in both seasons. However, during winter, juveniles (<7.9 mm) were found at the high- and mid-tide level, whereas during summer, juveniles were present at the mid- and low-tide level. During winter, no juveniles (<7.9 mm) and medium (8.0-17.9 mm) mudsnails were found at the low-tide level. However in summer, juveniles (7 individuals/m²) and medium (20 individuals/m²) were found at the low-tide level (Figure 3.9).

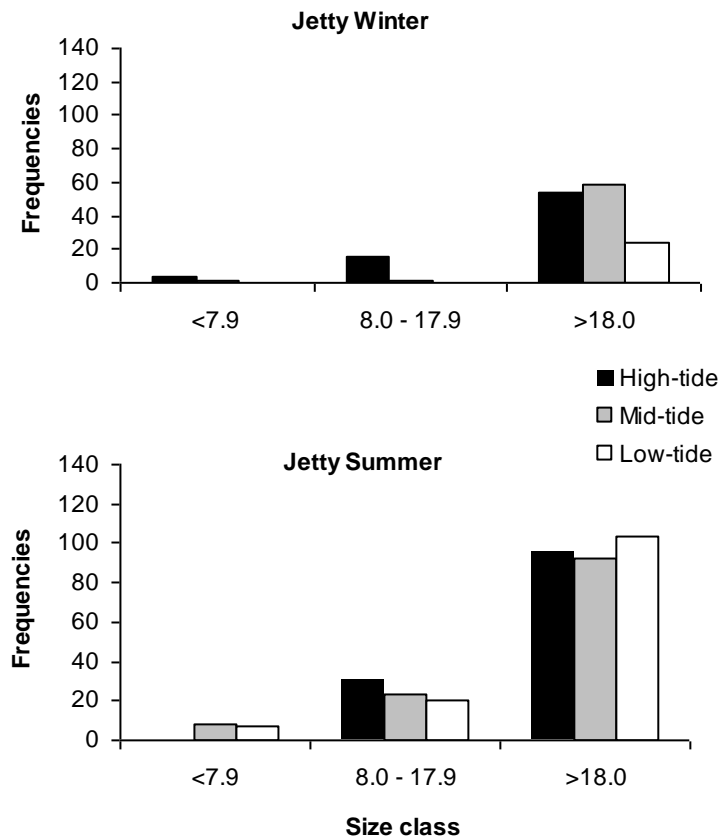


Figure 3.9 Number of individuals/m² within each size class at all tidal levels at the Pleasant Point Jetty site. Size classes as in Griffin and Thomson (1992).

d) Population structure at the Oxidation Ponds site

The mudsnail population at the Oxidation Pond site was dominated by smaller individuals (6-10 mm) at the high-tide level during winter and mid-tide level during summer. In contrast, mudsnails of shell length 21-25 mm dominated the low-tide level in both seasons. The highest number of juveniles (<5 mm) was found during summer at the mid-tide level (5.7%) compared to the high-tide level (1.4%) and the low-tide level (0%). In winter, juveniles formed 3.4% of the population at the high-tide level but no mudsnails were found at the mid- or low-tide level. No mudsnails of shell length 26-30 mm were found at the high- and mid-tide level in winter or summer, however, 3.4% of the populations were found during winter at the low-tide level (Figure 3.10).

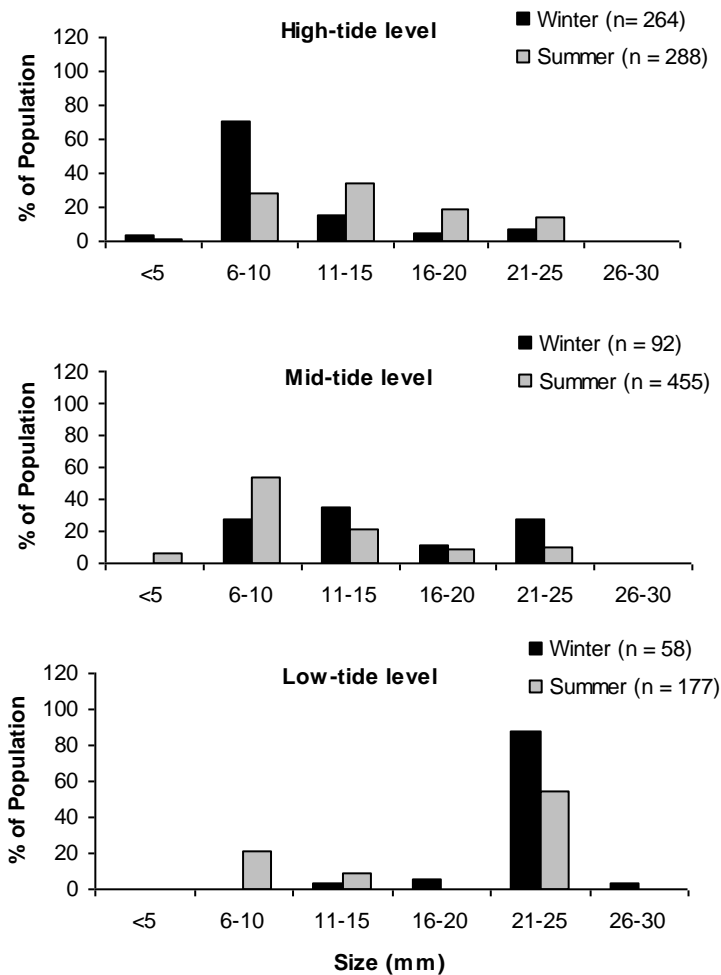


Figure 3.10 Oxidation Ponds. Seasonal population structure of *A. crenata* at all tidal levels.

Oxidation Ponds frequency distribution

At the Oxidation Pond, the medium size *A. crenata* class (8.0-17.9 mm) had high densities at the high- and mid-tide level during winter and summer. The highest number of juvenile *A. crenata* (<7.9 mm) was found at the mid-tide level (137 individuals/m²) during summer, compared to 6 individuals/m² during winter. No juveniles were found at the low-tide level during winter, whereas 16 individuals/m² were found during summer (Figure 3.11).

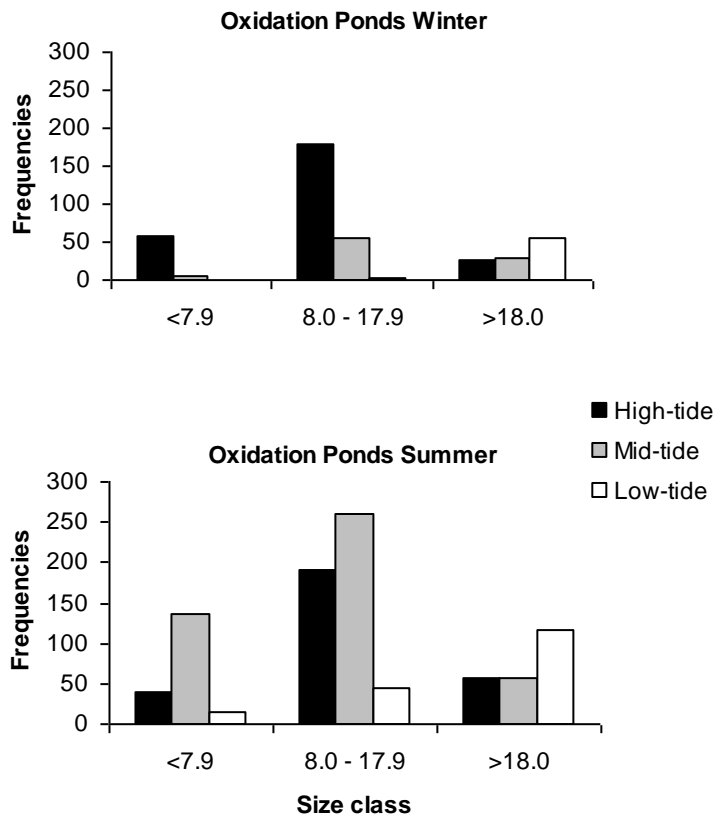


Figure 3.11 Number of individuals/m² within each size class at all tidal levels at the Oxidation Ponds site. Size classes as in Griffin and Thomson (1992).

e) Population structure at site Tern Street

At the high-tide level, the mudsnail population at the Tern Street was dominated by medium individuals (16-20 mm) which formed 77.7% of the population during winter and 82% during summer. At the mid-tide level, the highest population was dominated by mudsnails of shell length 21-25 mm (62.5%). In summer mudsnails of shell length 16-20 mm contributed 52.9% of the population and there were 45.7% of mudsnails of length 21-25 mm. During summer, at the low-tide level, 66.7% of the population was made up of individuals of 21-25 mm and 26.7% by 16-20 mm mudsnails (Figure 3.12).

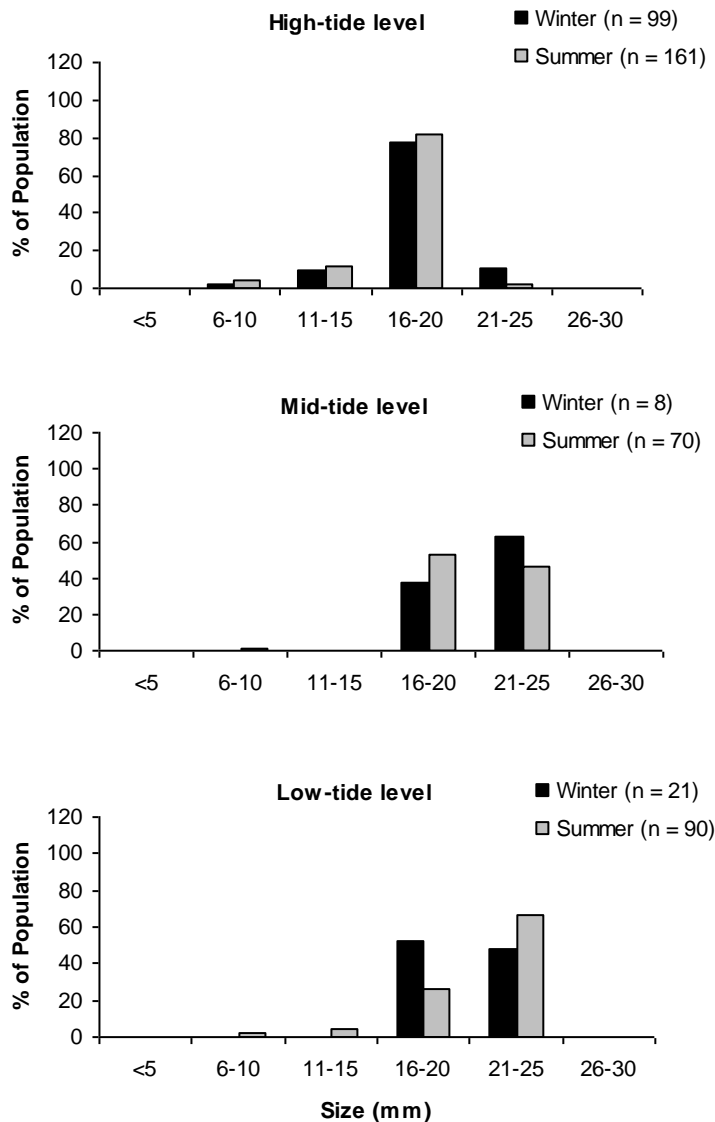


Figure 3.12 Tern Street. Seasonal population structure of *A. crenata* at all tidal levels.

Tern Street frequency distribution

The highest densities of adult (> 18.0 mm) *A. crenata* were found at the high-tide level during winter (63 individuals/m²) and summer (99 mudsnails/m²) at the Tern Street site. Medium mudsnails (8.0-17.9 mm) had very low abundance at the mid-tide and low-tide level during winter. No juveniles (<7.9 mm) were found at all tidal levels during winter, however, during summer 1 individual/m² was recorded at each level. During winter, 1 medium individual/m² was found at mid-tide level, however no medium mudsnail were found during summer (Figure 3.13).

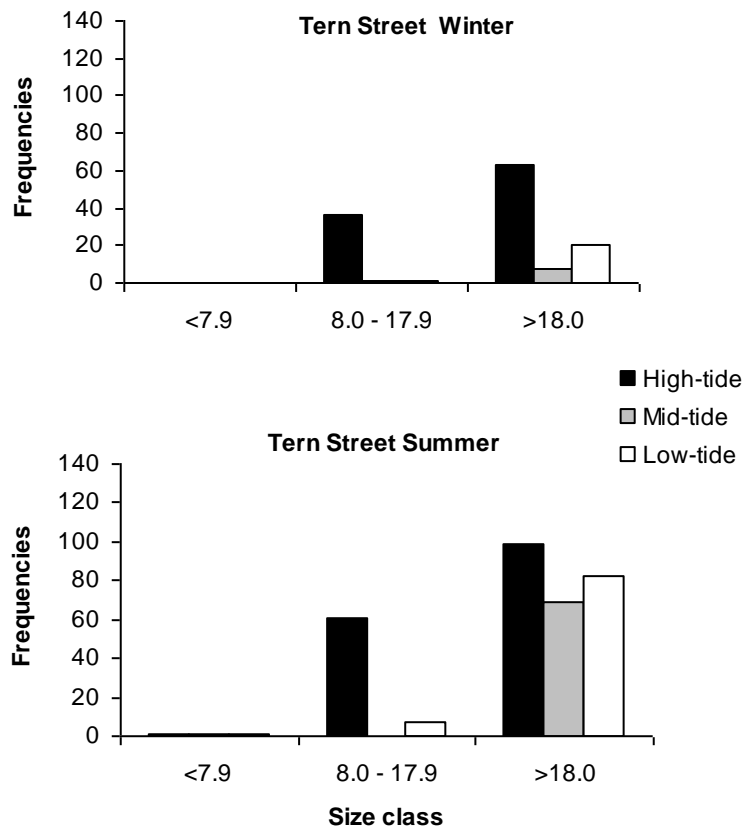


Figure 3.13 Number of individuals/m² within each size class at all tidal levels at the Tern Street site. Size classes as in Griffin and Thomson (1992).

3.4.3 *Amphibola crenata* biomass analysis

For each site the regression line relating shell length to dry weight in *A. crenata* was significant ($P < 0.001$), following the equation $Y = ax^b$ (Quinones and Michel-Morfin 2006) (See Table 3.3 for winter and summer at all sites). There was a good correlation between dry weight and shell length for all sites and seasons. When plotted on power model the relationship between dry weight and shell length was linear, indicating isometric growth. Figure 3.14 shows the predicted weights (calculated from the regression lines) for large (23 mm), medium (15 mm) and small (8 mm) individuals. The estimated dry weight of *A. crenata* showed no significant differences between sites, or between seasons for all size classes (Table 3.4). For large (23 mm) the predicted dry weight ranged from 2.5 g to 3.3 g in winter and 1.7 g to 2.6 g in summer. For medium (15 mm) the predicted dry weight ranged from 0.6 g to 0.7 g in winter and 0.5 g to 0.7 g in summer. For small (8 mm) the predicted dry weight ranged from 0.07 g to 0.08 g in winter and 0.06 g to 0.10 g in summer.

Table 3.3 Regression equations and correlation coefficients (r) relating dry weight of *A. crenata* to shell length in winter and summer at different sites. Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Site	Winter	r	Summer	r
AV	$y = 0.00109x^{3.2697}$	$r = 0.9804$	$y = 0.0011x^{3.2025}$	$r = 0.9741$
FM	$y = 0.00107x^{3.3617}$	$r = 0.9177$	$y = 0.0012x^{3.0202}$	$r = 0.9244$
JT	$y = 0.00109x^{3.2697}$	$r = 0.9804$	$y = 0.0012x^{2.8831}$	$r = 0.8217$
OP	$y = 0.00104x^{3.6150}$	$r = 0.9853$	$y = 0.00106x^{3.3824}$	$r = 0.9856$
TS	$y = 0.00105x^{3.4717}$	$r = 0.9549$	$y = 0.00107x^{3.3390}$	$r = 0.9809$

y = dry weight in gram
 x = shell length in mm

Table 3.4 Results of analysis of variance comparing the size classes of *A. crenata* between sites and seasons.

Size class	Source of Variation	SS	Df	MS	F	P-value
Large	Sites	0.630	4	0.157	1.929	0.270
	Seasons	0.530	1	0.530	6.498	0.063
Medium	Sites	0.014	4	0.004	0.782	0.591
	Seasons	0.007	1	0.007	1.488	0.290
Small	Sites	0.001	4	0.001	1.312	0.399
	Seasons	0.001	1	0.001	0.603	0.481

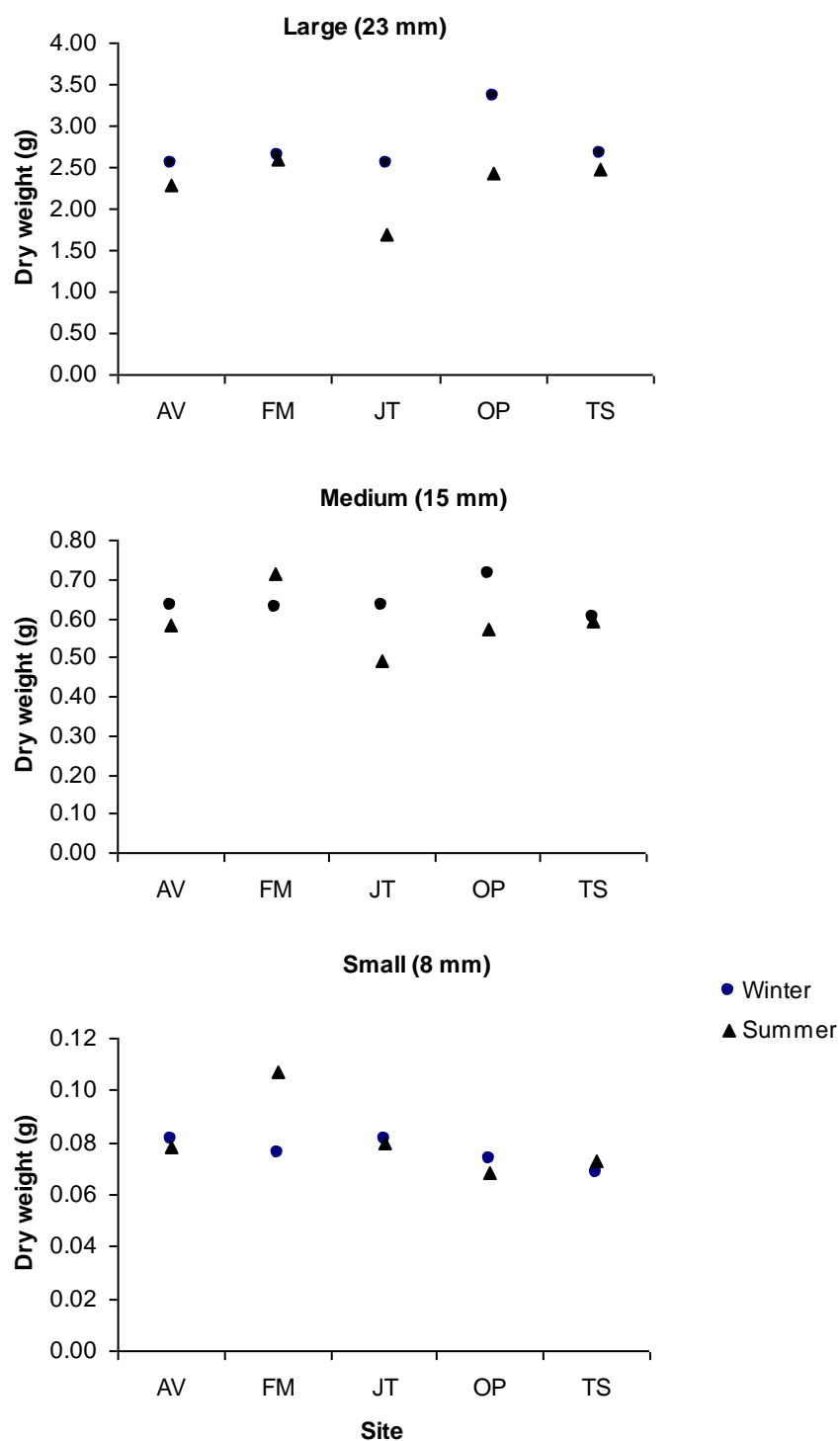


Figure 3.14 Predicted dry weights (g) of *A. crenata* for large (23 mm); medium (15 mm); and small (8 mm) in winter and summer at Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

3.4.4 Environmental parameter – organic content between seasons

In winter, the organic content of the sediment varied between sites (two-way ANOVA $F= 18.9$, $P< 0.001$) (Table 3.4). Ferrymead was significantly higher with the total mean of 2.3% and similar organic content was found at other sites ranging from 1.0% to 1.3% (Figure 3.15). However, there was no effect of tidal levels on the organic content in sediments (two-way ANOVA $F= 19.9$, $P< 0.001$). There was a significant interaction between sites and tidal levels on the organic content in sediments during winter (two-way ANOVA $F= 10.4$, $P< 0.001$) (Table 3.5).

In summer, there was no effect of site and tidal level on the organic content in the sediments. There was also no interaction between sites and tidal levels on the organic content in sediments during summer (two-way ANOVA $F= 1.9$, $P= 0.1$) (Table 3.5).

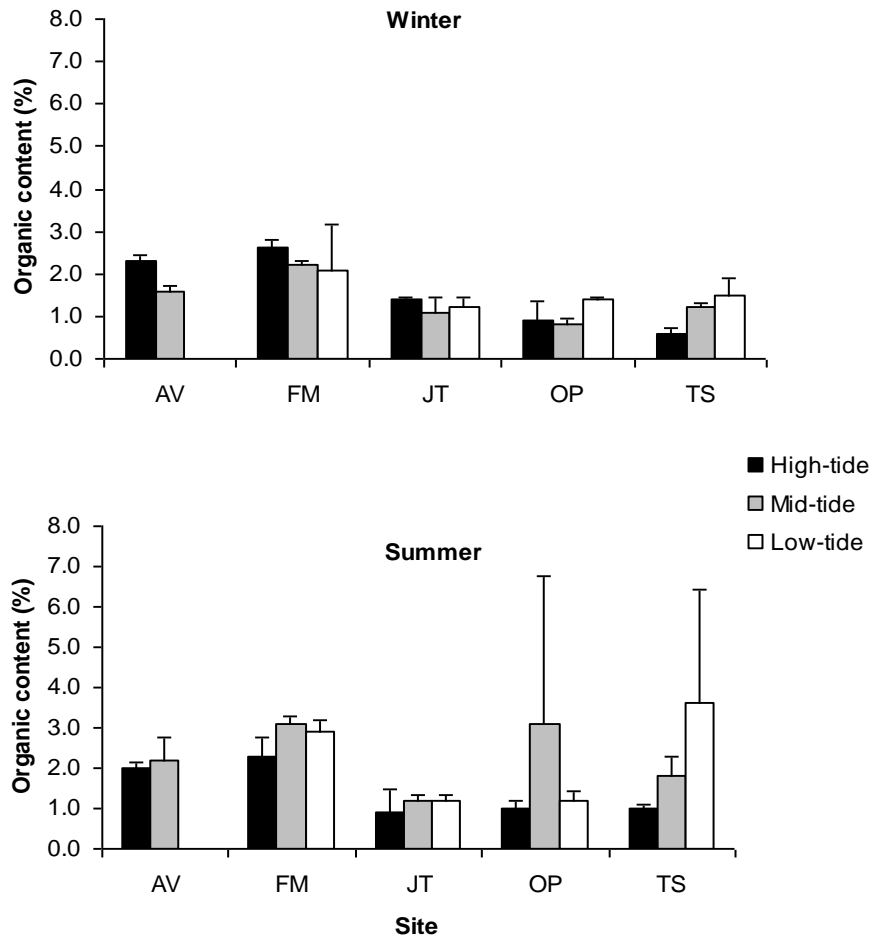


Figure 3.15 Organic content (% \pm SE) in the sediments at all tidal levels for winter and summer at Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Table 3.5 Results of analysis of variance comparing the organic content (%) between sites and tidal levels in winter and summer.

Seasons	Source of Variation	SS	Df	MS	F	P-value
Winter	Sites	9.408	4	2.352	19.895	0.001
	Tidal levels	0.707	2	0.354	2.991	0.065
	Interaction	9.797	8	1.225	10.359	0.001
Summer	Sites	15.105	4	3.776	2.488	0.064
	Tidal levels	5.408	2	2.704	1.781	0.186
	Interaction	22.903	8	2.863	1.886	0.100

3.4.5 Environmental parameter –porosity between seasons

In winter, porosity in the sediments varied amongst sites (two-way ANOVA $F= 27.8$, $P< 0.001$) and for different tidal levels (two-way ANOVA $F= 21.2$, $P<0.001$) (Table 3.5). Ferrymead was significantly higher with total mean of 26.5% and similar pore water at Oxidation Ponds, Pleasant Point Jetty and Tern Street with 22.7%, 22.9% and 23.0% respectively. Avon was the lowest with 17.2% of pore water. The highest pore water values were at the high-tide and mid-tide levels with total mean of 24.1% and 23.4% compared to the low tide 19.9% (Figure 3.16). There was a significant interaction between sites and tidal levels on the pore water during winter (two-way ANOVA $F= 44.1$, $P< 0.001$) (Table 3.6).

In summer, porosity in the sediments varied amongst sites (two-way ANOVA $F= 112.8$, $P< 0.001$) and tidal levels (two-way ANOVA $F= 26.3$, $P< 0.001$) (Table 3.5). Ferrymead and Tern Street were significantly higher with porosity values of 24.4% and 23.5% respectively. Similar porosity in the sediments (19.0% to 19.2%) was found at Pleasant Point Jetty and Oxidation Ponds while the Avon site was the lowest with 12.2%. The highest pore water was at high-tide and mid-tide levels with total mean of 20.34% and 21.1% respectively compared to low-tide 17.6% (Figure 3.16). There was a significant interaction between sites and tidal levels on the pore water during summer (two-way ANOVA $F= 40.2$, $P< 0.001$) (Table 3.6).

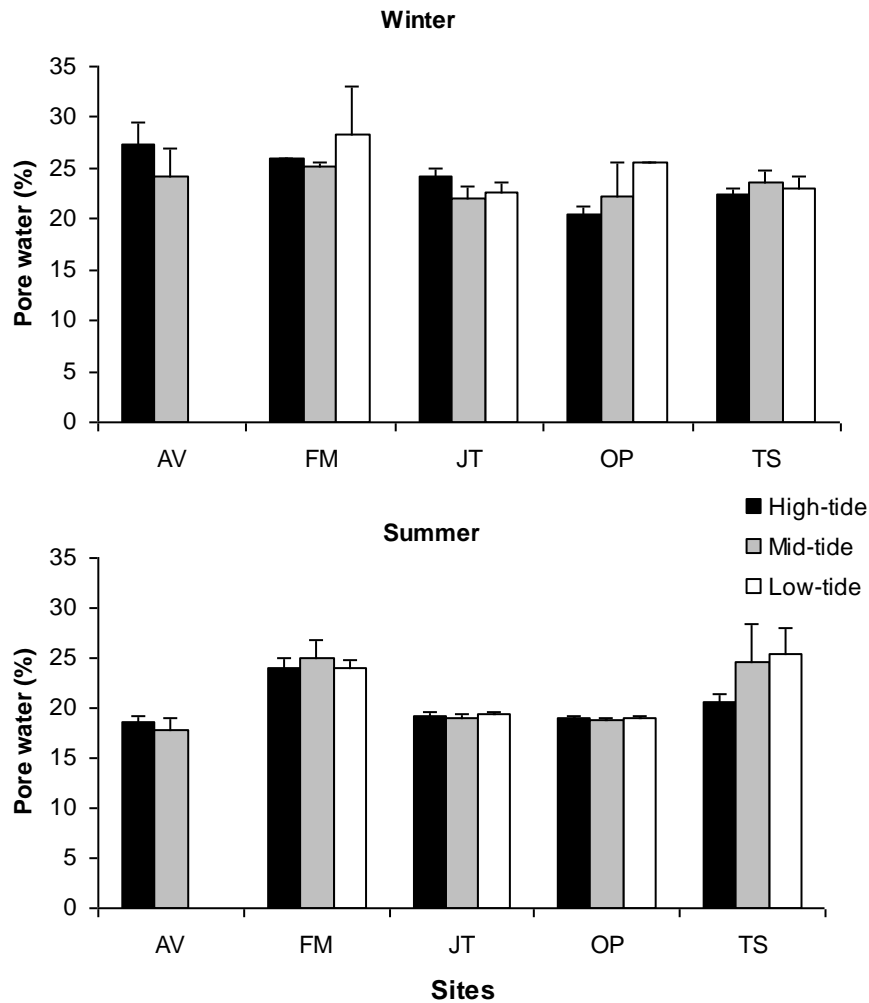


Figure 3.16 Pore water (% \pm SE) of the sediments at all tidal levels for winter and summer at Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Table 3.6 Results of analysis of variance comparing the pore water (%) between sites and tidal levels in winter and summer.

Seasons	Source of Variation	SS	df	MS	F	P-value
Winter	Sites	397.770	4	99.442	27.777	0.001
	Tidal levels	151.875	2	75.938	21.212	0.001
	Interaction	1262.338	8	157.792	44.076	0.001
Summer	Sites	853.403	4	213.351	112.791	0.001
	Tidal levels	99.575	2	49.788	26.321	0.001
	Interaction	607.592	8	75.949	40.152	0.001

3.5 Discussion

Density, size distributions and biomass of *Amphibola crenata*

A. crenata was broadly distributed at all sampling sites across the estuarine intertidal zones indicating that it can tolerate a wide range of environmental conditions (Bennington 1979). All size classes of *A. crenata* are tolerant to combinations of factors such as temperature, salinity, and oxygen concentrations (Shumway and Marsden 1982).

The present study found that larger *A. crenata*, 11-20 mm shell length inhabited the low-tide level. The wider distribution of large *A. crenata* at this level may be due to active or passive migration of this size class to the adjoining mudflat areas prior to breeding and egg laying (Briggs 1972). Mudsnaills with a shell length less than 10 mm were common at high-tide level rather than mid- and low-tide levels. This may be due to the inability of smaller *A. crenata* to maintain low respiration rates when submerged (Shumway and Marsden 1982).

The results of this study also showed that the size of *A. crenata* varied with tidal level. Watters (1964) found similar results for Hoopers Inlet, Otago. Later, studies by Bennington (1979) also showed that smaller sizes of *A. crenata* decreased in density towards low-tide level while adults' *A. crenata* increased in density from the high- to the low-tide level.

Densities of *A. crenata* in July 2009 were highest at the Oxidation Ponds with a density of 84 individuals/m² (combined all tidal levels). Studies by Bennington (1979), which were carried out in June 1975, also found a high density of 175 individuals/m². In the present study, 219 individuals/m² with shell lengths less than 10 mm were found in July which was half that recorded in June 1975 (420 individuals/m²). This area was favoured for larval settlement because of the unique combination factors such as particle size, nutrient levels, salinity range, and tidal exposure time (Bennington 1979). For adult *A. crenata* with shell lengths 21-30 mm, 98 individuals/m² were found in July 2009 compared to 21 individuals/m² in June 1975. Thus, at present, this site supports higher juvenile compared to adult individuals. The sparse distribution of large adults in this site may be due to the

stressful conditions caused by toxic substances from the sewage effluents but suitable for veliger settlement (Bennington 1979).

At Pleasant Point Jetty, *A. crenata* the 21-25 mm shell length contributed the highest proportion of the population during winter or summer at all tidal levels (Figure 3.8). There were no seasonal trends amongst this size class of *A. crenata* at this site, which was a similar finding to Bennington (1979). *A. crenata* with a shell length less than 10 mm occurred in July 2009 with 8 individuals/m² and similarly, Bennington (1979) found 10 individuals/m² during June 1975. Later, the same size class increased in density to 28 individuals/m² in December 2009, however none were found in Bennington's findings in December 1975. This difference might be due to better conditions in the estuary at present or differences in settlement patterns between years. Studies on the nidus formation showed that the peaks in egg masses restricted to a particular breeding season and similar studies found in intertidal pulmonate gastropod, *Salinator solida* (Roach 1996 cited in Roach and Lim 2000).

A. crenata with a shell length less than 10 mm was found (12 individuals/m²) in December 2009, however, Bennington (1979), found three times this density in December 1975. Densities for medium (11-20 mm) and large (21-30 mm) *A. crenata* were similar to Bennington's (1979) findings in December 1975. The sediment at the Avon site was very soft mud at the mouth of Avon River and this might favour small *A. crenata* with shell length less than 10 mm. In addition, higher densities of this shell length *A. crenata* occurred at high-tide level compared to other sites in December 2009. Juvenile mudsnails may prefer the high-tide level or more exposed upper shore due to their inability to maintain low respiration rates when submerged (Shumway and Marsden 1982).

At Ferrymead, the total densities / m² were less in 2009 than in 1975. There were fewer small and medium individuals, however the number of large individuals (21-30 mm) had increased from 25 individuals/m² to 48 individuals/m². This might be due to slower growth rate of the smaller size classes and apparently stationary cohorts of larger individuals. The fine clay sediment is not suitable for juvenile *A. crenata* and might affect the growth rate of

smaller and medium size individuals. Besides, this site is exposed to human disturbances because it is situated near to the walking track. Also, on the opposite side of the Heathcote River is the Ferrymead Heritage Park. Large numbers of people walk in these areas and this might affect the survival of smaller individuals. Trampling by humans can modified the abundance and population dynamics of the clam *Macoma balthica* and the cockle *Cerastoderma edule* (Rossi *et al.* 2007).

At Tern Street, small *A. crenata* less than 10 mm were found in low numbers (2 individuals/m²) in July 2009 and none were found in December 2009. Throughout the year, *Zostera novazelandica* (sea grass/ eel grass) dominated the low-tide level and this might have contributed to the low density of juveniles (< 10mm) at this site. *Zostera novazelandica* was abundant in the estuary during 1920s and disappeared in 1950s (Jones and Marsden 2005). Sea grass production depends on tidal height and there is high biomass (518g dry weight per m²) near the low-tide channels (Jones and Marsden 2005). This site is also located near a dense residential area and human access to this area might be considered a factor affecting the survival of smaller *A. crenata*. The firm sand sediment also may not be favourable for *A. crenata* settlement.

While the density of *A. crenata* was variable in winter it increased in the summer at all sites. During winter, *A. crenata* tended to burrow in the sediments and fewer individuals remained active on the mudflat surface. Similar findings have been found in other species, for example, *Salinator solida*, which buried in the sediment and possibly affecting the accuracy of a census (Roach and Lim 2000). However, in the present study, the sediment was dug and sieved hence this was not a factor contributed to the lower density recorded for *A. crenata* in the winter. Seasonal migration might be responsible for the changes in abundance and size structures which were observed at all sites. Seasonal fluctuations in distribution are common among intertidal gastropods (Gendron 1977). Also, environmental conditions such as sediment type, temperature, and salinity might influence the density of *A. crenata* in the Avon-Heathcote Estuary.

At Pleasant Point Jetty, the predicted dry weight for mudsnails of a shell length of more than 10 mm was 2.2 g and this was similar to findings by

Bennington (1979) who found a mean dry weight was 2.4 g. He showed that there were no significant differences between sites or months for mudsnails greater than 10 mm in December 1976, and this was similar to results for medium and large size classes of *A. crenata* at Pleasant Point Jetty. In the present study, at the Avon site, the total dry weight was 1.22 g, with individuals 7 to 18 mm making up the largest contribution. However, in December 1976, the total dry weight was less than 1.0 g mostly of individuals of less than 10 mm shell length (Bennington 1979). At Ferrymead, the total dry weight biomass per m² was 3.91 g which comprised individuals from 4 mm to 21 mm. However, Bennington found much higher biomass of more than 10 g made up by 10 – 19 mm individuals in December 1976.

Environmental parameters in the Avon-Heathcote Estuary

Organic content in the sediments varied between sites. The highest organic content was found at Ferrymead which recorded above 2% at all tidal levels. This result contrasted with Bennington (1979) who recorded 1-1.75% organic content in June 1975 at the same site. The organic content at Pleasant Point Jetty ranged from 1.1%-1.4% in the present study and was similar to Bennington (1979) who recorded 1.0-1.75% at Pleasant Point Jetty in June 1975.

Sediment particle size can also determine the percentage of organic content in sediments. Although, in the present study correlations between sediment type and organic content were not undertaken, there are numerous studies supporting the positive relationships between these two factors. Robertson *et al.* (2002) confirmed that Ferrymead sediments consisted of fine silts or clay sediment and these are generally associated with a high organic content (Bolton-Ritchie 2008). In addition, this site, which is situated at the mouth of the Heathcote River, might accumulate higher levels of organic matter compared with other sites. Organic matter was also high at the mouth of the Avon and Heathcote Rivers (Voller 1973). Contrasting with this, Tern Street was the site with the lowest organic content. This may be due to the composition of sediment that is made of firm mud

or sand. This result was similar to McConway (2009) who found that organic matter content was low at Tern Street.

Pore water values in the sediments were higher in winter than in summer 2009. During each tidal cycle, the high- and mid-tide levels are exposed to sunlight for longer than the low-tide level. This allows the water between the particles to evaporate faster at the higher-tidal levels. Evapotranspiration rate is higher on the mudflat Gazi Bay, Kenya compared to the *Sonneratia alba* stand. This leads to higher temperatures and wind speed on the mudflat which affects the distribution patterns of *Terebralia palustris*, a common gastropod found at Gazi Bay, Kenya (Pape *et al.* 2008).

3.6 Conclusions

Densities of *Amphibola crenata* were higher in summer compared to winter. Seasonal migration, as well as the unique environmental conditions at each site, might contribute to seasonal fluctuation of *A. crenata*. Distributions of *A. crenata* were broadly distributed at all tidal levels with the size of *A. crenata* increasing at lower tidal level at all sampling sites. Juveniles prefer to inhabit the high-tide level because of finer sediments which favour settlement at this level. Medium and adult *A. crenata* were common at mid-tide and low-tide levels because of their high salinity tolerance. There was a good correlation between the dry weight and shell length for all sites and seasons. The dry weight to length relationship of *A. crenata* showed no significant differences between sites or seasons for all size classes. The organic content in the sediments varied among sites consistent with sediment particle size.

Because *A. crenata* abundances vary between sites and seasons it is important that future studies take these variables into account when following long- or short term population changes. The sites selected for this study form part of a long term study to evaluate the effects of changed nutrient conditions on the abundance and biomass of key estuarine organisms. The present research which

demonstrated that the population of *A. crenata* varied between sites supports the use of this species as an indicator of estuarine change.

Chapter 4: CAGE EXPERIMENT

4.1 Introduction

Growth rate of intertidal grazing gastropods can vary with many environmental and internal factors (Paine 1969; Sutherland 1970; Lewis and Bowman 1975; Creese 1980; McCormack 1982; Fletcher 1984; Underwood 1984). For example growth rates of intertidal limpets can be affected by factors such as microhabitat, tidal elevation, wave action, algal food availability and intraspecific or interspecific competition (Branch 1981). Gastropods are mobile animals and thus can experience several type of habitat during their life history (Paine 1969; McQuaid 1982). One common way of investigating differences in growth rate between habitats is to mark individuals and track their growth rate periodically (Takada 1995).

Benthic microalgae

In estuaries or intertidal mudflats without macroalgae or seagrass, primary production is due mainly to surface microbenthos (Yallop *et al.* 1994; MacIntyre and Cullen 1995). This provides the food source for *Hydrobia* species (Forbes and Lopez 1990) and also for the New Zealand pulmonate snail *Amphibola crenata*. The food availability (microalgal abundance) can affect the growth rate, density, and competition among grazing gastropods (Branch and Branch 1980, Underwood 1984) and this factor varies with season and tidal level.

The primary productivity of the microphytobenthos can be influenced by environmental and seasonal factors which include temperature, light, exposure and duration of sunlight, nutrient availability, sediment type, tidal and diel rhythms (Perkins *et al.* 2003; Cook *et al.* 2004; Underwood and Kromkamp 1999). In summer or spring, the primary productivity of the microphytobenthos is higher because of high temperatures and sunlight exposure on the sediment surface during low tide. Muddy sediment areas can also be high in primary production where the sediment is stable and there is little wave action (MacIntyre *et al.* 1996; Underwood and Kromkamp 1999). Bacillariophyceae or motile diatoms

dominated the intertidal mudflats (Serodio *et al.* 1997) followed by flagelletes-dinoflagellates and euglenophytes (Blackburn 1987) and cyanobacteria-blue-green algae (Reise 1992).

In estuarine sediments, the algal biomass can be estimated using the proxy of chlorophyll *a* concentration (Perkins *et al.* 2003). About 50% of primary production is produced by microphytobenthos from the top 2 mm of sediment surface (De Jonge and Van Beuselum 1992). The oxygen and nutrient availability decrease with increasing sediment depth and this can cause anoxic conditions which inhibit the microphytobenthos (Admiraal 1984; MacIntyre *et al.* 1996; Consalvey *et al.* 2004).

The main pigment of microalgae is chlorophyll *a* which can absorb light in the red (650-700 nm) and blue (400-450 nm) spectrum (Kirk 1994; Consalvey *et al.* 2004). Other chlorophyll pigments such as chlorophyll *b* and *c* are also present in microalgae and these can absorb a wider spectrum of wavelength increasing the chance and rate of photosynthesis (Consalvey *et al.* 2004). Chlorophyll *b* occurs only in green algae while chlorophyll $c_1 + c_2$ can be found in the members of the chromista and dinoflagellates. Both chlorophyll pigments can be used as indicators of taxonomic change in the microphytobenthic communities (Geider and Osborne 1992).

Chlorophyll pigments can be collected from benthic sediments using various methods, for example using mini-cores, contact cores or sections frozen in situ using a Cryolander (Perkins *et al.* 2003). Microphytobenthos can also be observed by changes in sediment colour. For example, green patches can be found on the surface of sediment when *Euglena* or allied genera are present in high abundance, especially where areas are polluted or the salinity is low (Day 1981). The development of biofilms of various green, browns and gold depend on which taxa of benthic microalgae are available on the mudflat surface during the daylight hours of low tide (Round and Palmer 1966; Cane 1996). McClatchie *et al.* (1982) identified 64 diatom species in the Avon-Heathcote Estuary and later Cane (1996) recorded 66 taxa of microalgae. Variations of pinnate diatoms, euglenoids,

flagellates and blue-green algae have also been found in the Avon-Heathcote Estuary (Bruce 1953; Williams 1960; Knox 1992).

Nutrient enrichment in the estuaries

Nitrogen and phosphorus are generally the limiting nutrients for plant growth and determine primary productivity (Diaz and Rosenberg, 2008). However nutrient enrichment in marine waters can cause excessive growth of algae such as sea lettuce, *Ulva* spp. The abrupt increase in macroalgae can be referred to as a bloom and this can result in eutrophication. Limited nutrient enrichment can result in an increase of submersed microphytes whilst higher concentrations results in the dominance of phytoplankton, filamentous algae or floating plants and this is followed by a decline in submersed macrophytes (Thomas and Daldorph 1994). Aquatic macrophytes can benefit pulmonate snails (Bronmark 1985; Thomas and Tait 1984; Underwood 1991) and the disappearance of submersed macrophytes can result in a decline in snail populations. Under eutrophic conditions macroalgal biomass can build up and form aggregates that sink and fuel bacterial growth in bottom waters and sediments. Eventually microbial decay of these blooms increases the availability of organic material and nutrients and enhanced microbial respiration decreases the dissolved oxygen (DO) levels, altering the underlying trophic status (Barnes and Hughes 1982; Bressington 2003).

Since 1882 Christchurch City Council has released its treated municipal wastewater into the Avon-Heathcote Estuary. The Christchurch population is projected to increase to 420,000 people in 2050 with the predicted wastewater inflow to the treatment plant of 230,000 m³/day, and peak wet weather flow of 690,000 m³/day (Miller *et al.* 2004). Discharge from the wastewater treatment plant accounted for around 80-90% of all nitrogen loading and 94-98% of all phosphorus loading into the estuary (Jones and Marsden 2005; Walrond 2007). Wastewater-derived ammonium ions dominate the dissolved inorganic nitrogen nutrient input to the estuary and come almost exclusively from the wastewater (Bolton-Ritchie and Main 2005).

Due to diversion of sewage through ocean outfalls in the Avon-Heathcote Estuary, researchers anticipated that nutrient enrichment due to loss of primary nutrient source will affected the biomass and productivity of the primary producers as well as modified the population structure, density and distributions of *A. crenata*.

Effects of trace metals on organisms

Copper, lead and zinc are the most common contaminants found in estuaries and are also major components of urban runoff (Roper *et al.* 1995). In marine invertebrates, uptake of dissolved trace metals occurs via the food supply (Furness and Rainbow 1990; Rainbow 1993) and from solution. Factors such as species, food type, concentrations of metal in the water and physicochemical parameters of the water determine the priority of the uptakes (Rainbow 1993). Fluctuating salinity conditions in the estuary allow zinc and cadmium to be more bioavailable at lower salinities for invertebrates (Furness and Rainbow 1990).

Many marine organisms have the potential to bioaccumulate high levels of metals from their environment (Fowler 1990; Phillips and Rainbow 1994; Szeffer *et al.* 1999). Benthic communities are often exposed to effluent discharged from numerous anthropogenic sources including solid waste disposal (Amin *et al.* 2009). Trace metals are effectively partitioned onto estuarine intertidal sediments because of their high organic content and pH (Luoma *et al.* 1990). Elevated trace metals in the sediments might cause an ecotoxicological threat to macrobenthic invertebrates (Amin *et al.* 2009). Gastropods are potential indicators of environmental stress through changes in diversity and community structure due to the chronic and acute effects of sediment contamination by trace metals (Traunspurger and Drew 1996).

Environmental conditions can determine the structure of adult populations by affecting the survival of newly settled juveniles. Metals can also delay settlement and burrowing of estuarine invertebrates (Furness and Rainbow 1990). Copper and zinc caused *Macomona liliana*, a deposit feeding bivalve, to drift away from increased trace metal contamination thus changing the distribution of

this species. A wide range of environmental factors can influence the uptake and toxicity of trace metals in organisms (Kennish 1992).

4.2 Objectives

The aims of this study were to:

- 1) Measure the growth rate and condition index (health) of *A. crenata* from five sites with different contamination and nutrient levels
- 2) Identify spatial and temporal variation of the benthic microalgae which is the food source for *A. crenata* (measured as sediment chlorophyll *a*, *b* and *c* concentrations) between sites;
- 3) Experimentally investigate the relationship between the growth rate and condition index of *A. crenata* in relation to food sources between sites; and
- 4) Measure changes in nutrient levels (total nitrogen, phosphorus and trace metals) in the sediment and water between sites.

4.3 Methods

4.3.1 Location of study sites

Five sites were selected for the cage experiment. They were 1) Pleasant Point Jetty; 2) Tern Street; 3) Oxidation Ponds (200m south of main outfall); 4) Kibblewhite Street – Avon river mouth (control site) and 5) Ferrymead – Heathcote river mouth (control site). The sites were chosen based on the availability of *A. crenata* population which consisted of a good size range of individuals. Other factors such as differences between sediment types, influence of freshwater, proximity of nutrients inputs and metal concentrations were also taken into account.

4.3.2 Cage Experiment

The dimension of the cage was 38 cm long, 27 cm width and 12 cm height (Fig. 4.1). Six plastic cages were moored at each site, three experimental cages containing *A. crenata* populations and three empty cages as controls. Cages were moored from 1st November until 7th November 2009. The cages and mudsnails were retrieved six weeks later from 14th December until 18th December 2009. The cages were checked every week to remove any algae or animals trapped on the outside.

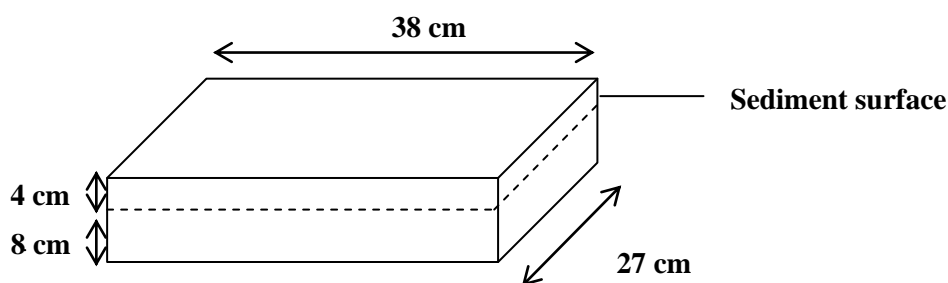


Figure 4.1 Dimension of cage used in the field experiment.

From each site 75 mudsnails were collected, 30 juveniles with an apex to whorl length less than 7.9 mm, 30 medium that ranged from 8.0-17.9 mm, and 15 adult mudsnails larger than 18.0 mm. The wet weight of each snail was recorded using a field electronic balance. The mudsnails were painted using Dulux spray paint (blue and pink; blue and green; and blue) with a unique code (Appendix 2) in order to track the growth of snails at the end of the six weeks. Dulux spray paint was used for this experiment as in Swinscoe (2008). All the snails were left to dry about 4 hours in the sun.

In the meantime an area was marked out at the high water level. Because some of the sites were also being used by staff at NIWA, at these locations a new area was established 2.5 m down shore of NIWA's mark or sticks. A 30 m by 2.5 transect was established by running a tape parallel to water line (Figure 4.2).

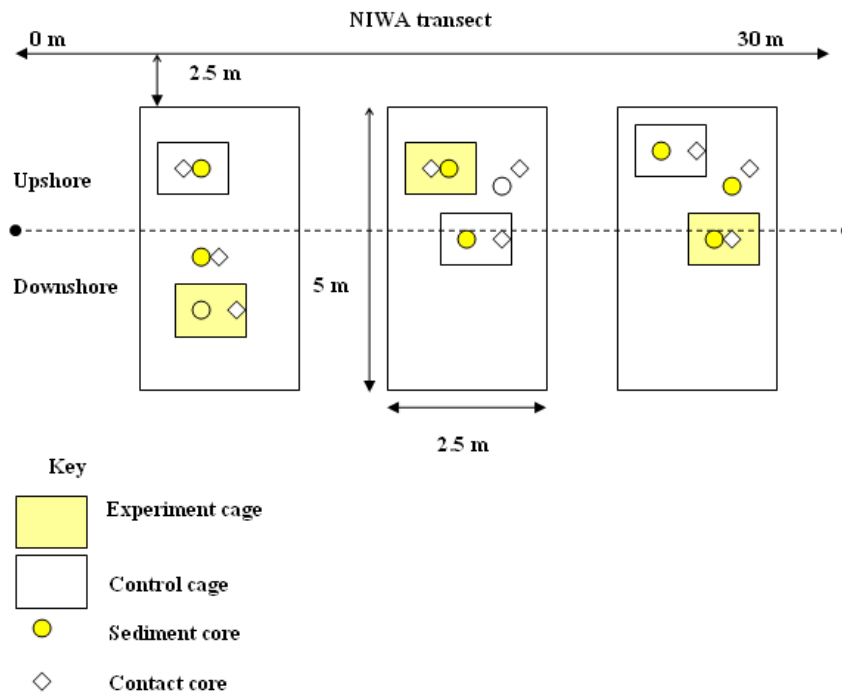


Figure 4.2 Layout of the transect and position of experimental and control cages, sediment core and contact core at each site.

A set of random numbers from 1 to 30 was generated using Microsoft Excel. The numbers were used to determine the positions of the cages (Figure 4.2). The sediment was dug using a shovel to ensure that the cage was semi-buried with 4 cm of the upper cage left above the sediment surface (Figure 4.1). The cage was pressed into the sediments and prongs were placed at both sides of the cage so that the cage would not float away during high tide (Figure 4.3). Later, 25 painted mudsnails (5 large, 10 medium and 10 juveniles) were placed in each experimental cage through the small opening on the top (Figure 4.4). Finally, the opening was closed using metal ties to ensure no mudsnails would escape or float during high tide.

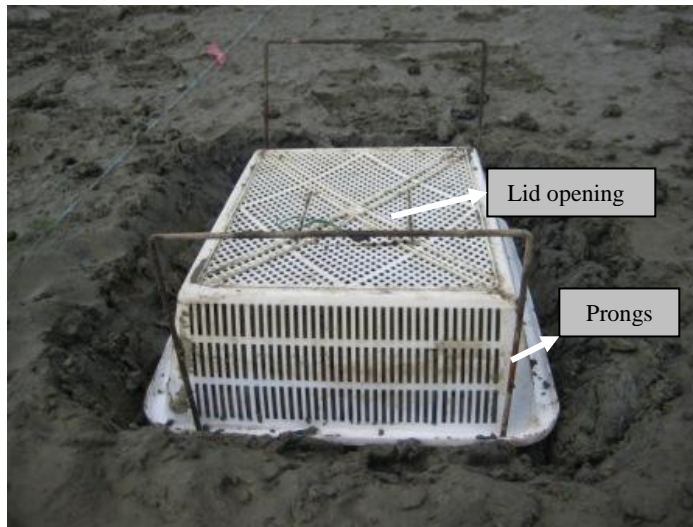


Figure 4.3 Cage used in the field experiment showing prongs to keep it in place.



Figure 4.4 Painted *A. crenata* in the experimental cage.

4.3.3 Collection of sediment samples for chlorophyll content determination

Sediments were sampled using a contact corer, 48 mm in diameter and 7 mm in depth (Figure 4.5).

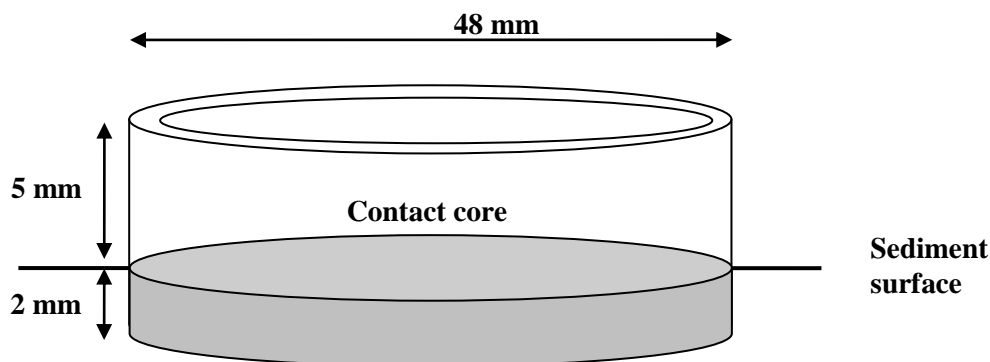


Figure 4.5 Diagram showing the metal contact core.

One sediment sample was collected within each experimental and control cages and also one just outside of each cage. The contact corer was placed onto the sediment surface with the shallow 2 mm dish against the sediment surface. A small amount of pressure was used to push the corer deep enough to make contact with the sediment surface but not as much as to disturb sediment layers. The upper exposed dish was then filled with liquid nitrogen freezing the sediment below. Once the sediment had frozen the corer was lifted from the sediment surface taking with it a disk of frozen sediment. A knife was used to remove any excess sediment. The sediment was wrapped in tin foil and labeled with site name, cage, and date. The cores were kept frozen in liquid nitrogen in the thermos flask and transported back to the laboratory's freezer at -80°C and stored in ice cream container and analysed for chlorophyll content using spectrophotometry.

4.3.4 Collection of sediment samples for total phosphorus, nitrogen and trace metals determination

A corer of 5 cm in diameter and 7cm of depth was used to obtain sediment samples for trace metal content. From each cage, a sample of sediment was collected and was placed in 250 ml plastic bottle container labeled with location, cage and date of collection. All the samples were stored in a chilly bin with ice

before transported back to laboratory. Later the samples were kept at 4 °C before further analysis.

4.3.5 Collection of water samples for total phosphorus, nitrogen and trace metals determination

Polyethylene 100 ml bottles were used to collect the water samples. Each of the bottles was washed with hydrochloric acid (5%) solvent, distilled water and Milli-Q water 3 days prior to experiment. On day one, the clean bottles were filled with hydrochloric acid (5%) solvent using a beaker. The clean lids were put into Agee jar and covered with hydrochloric acid (5%) solvent and were left overnight. On day two, the hydrochloric acid (5%) was poured back into the beaker and all bottles were filled with overflowing distilled water. The lids were also put into the Agee jar filled with distilled water and were left overnight. On day three, all the bottles and lids were filled with 1/3 full of distilled water and rinsed twice. Bottles were also rinsed three times with Milli-Q water. The bottles were shaken as much as possible to excrete water and caps were screwed instantly.

In the field, water samples were collected from the nearest puddles of water using a 60 ml syringe. All the bottles were labeled with location and date. All the samples were stored in chilly bin with ice before transported back to laboratory. Later the samples were kept in -80°C freezer before further analysis.

4.3.6 Growth rate and Condition Index (CI) of *A. crenata*

Surviving mudflat snails from the cages were collected from each of the experimental cage. All snails were put in ice-cream containers covered with old newspaper, rinsed with sea water and left to dry with tissue paper. Next, their shell lengths (mm) were measured with electronic calipers. Their wet weights (g) were also recorded using a balance to three decimal points. All snails were wrapped in tin foil labelled with a code indicating the site, cage and individual number. They were dried in a tray in 65 °C oven for 72 hours. The dry weight (g) of each individual was recorded.

The growth was calculated as below:

Shell growth = length (mm) = Shell length at end of experiment - Shell length at the start

Body weight (g) = Wet weight at final - Wet weight at the start

Dry weights (g) of individual snails were recorded using a balance to three decimal places. Foils were removed and each individual was placed in crucible jar with code. Twenty five snails were put in the ashing oven per time and were ashed at 450 °C for 4 hours. Using gloves, the crucible jars with snails were removed from the ashing oven using a thong and left to cool. Once cool, the individual snails were removed from the crucible jars and shells were tapped to remove ash (organic sediment). Ashed free dry weights (g) of individual snails were recorded using a balance to three decimal points. Condition Index (CI) was calculated as below:

$$\text{Condition Index (CI)} = \frac{\text{Dry flesh weight (g)}}{\text{Dry shell weight (g)}}$$

4.3.7 Spectrophotometric measurement of sediment chlorophyll content

Preparation of the sediment

Cores were removed from -80 °C freezer and samples were placed in chilly bin with dried ice. Samples from two sites were processed at each time. Using the vernier calipers, the cake depth was measured at three different places to provide average. The whole cake was placed on the balance and the weight (g) was recorded to three decimal points. Small zip locked bag were labelled with number and one third of each cake was put in the zip locked bag. The entire zip locked bag was placed in the ice cream container and put into the chilly bin with dried ice and transported to National Institute of Water and Atmospheric Limited (NIWA) for analysis.

At NIWA, the zip lock bags were unsealed and put into the polystyrene tray and placed in the freeze dryer for 12 hours or overnight. After 12 hours, each

zip lock bag that contain one third of the cake was put in the chilly bin with ice for weighing. A beaker and centrifuge tube were placed on the balance and tared. A piece (1 g) of the core was broken off and placed into the 10 ml centrifuge tube, labelled and weighed (g). The weight was recorded into Excel program which connected the balance to the computer. Finally, all the centrifuge tubes were placed in the -80 °C freezer until further extraction.

Pigment Extraction

Wearing gloves, safety glasses and ear plugs for sonication process, a flask containing 90% acetone (extracting solvent) with attached of measuring pipette of 5ml was placed in the chilly bin with ice. Then 5 ml of 90% acetone was pipetted into the centrifuge tube. The centrifuge tube was held so that the probe was dipped below the solution surface of the sonication. The sonication probe was cleaned via submergence in a flask of water with ice and wiped each time. The gloves were changed regularly to avoid contact with the skin. Using the ear plugs, the content was subjected to 15 pulses to homogenize the mixture and broke the benthic microalgae cells (BMA). All samples were placed in the 4 °C fridge for 4 hours and shaken every hour to prevent extraction slowing prior to analysis. All the centrifuge tubes were placed in the Eppendorf centrifuge 5810 machine at speed of 3000 rpm/rcf for 10 minutes.

Spectrophotometric determination of chlorophyll concentration

The spectrophotometer was switched on thirty minutes prior to use. Samples were kept on a rack and covered with a towel to provide darkness to prevent further extraction. An empty cuvette provided a blank measurement whilst the laboratory cuvette was provided with a chlorophyll *a* reading. A new pipette was used for every sample and the cuvette was washed with acetone before each new sample. The supernatant containing the previously extracted chlorophyll pigments in each centrifuge tube was removed using a pipette. This was put into a cuvette until it was three quarter filled being careful to avoid picking up sediments. The cuvette was wiped with a tissue for each sample to avoid any

smears on the reading. The cuvette was always placed in the same direction in the spectrophotometer so that any scratches on the tube equally influenced every reading. In each session, 30 samples were analysed. A reading was taken for every sample. The data were recorded and calculations were carried out separately for chlorophyll *a*, *b* and *c* concentrations.

Chlorophyll content and concentrations ($\mu\text{g g}^{-1}$) were calculated from the spectrophotometry results using equations as outlined in Jeffrey and Humphrey (1975).

The equations are as below:

$$\text{Chlorophyll } a = \frac{[11.85(\text{SLW}_{664} - \text{SLW}_{750}) - 1.54(\text{SLW}_{647} - \text{SLW}_{750}) - 0.08(\text{SLW}_{630} - \text{SLW}_{750})] * \text{Ve}}{\text{weight of sample (g)}} \quad (\mu\text{g g}^{-1})$$

$$\text{Chlorophyll } b = \frac{[-5.43(\text{SLW}_{664} - \text{SLW}_{750}) + 21.03(\text{SLW}_{647} - \text{SLW}_{750}) - 2.26(\text{SLW}_{630} - \text{SLW}_{750})] * \text{Ve}}{\text{weight of sample (g)}} \quad (\mu\text{g g}^{-1})$$

$$\text{Chlorophyll } c = \frac{[-1.67(\text{SLW}_{664} - \text{SLW}_{750}) - 7.60(\text{SLW}_{647} - \text{SLW}_{750}) + 24.52(\text{SLW}_{630} - \text{SLW}_{750})] * \text{Ve}}{\text{weight of sample (g)}} \quad (\mu\text{g g}^{-1})$$

Key:

SLW 750 – Blank

SLW 664 – Chlorophyll *a*

SLW 647 – Chlorophyll *b*

SLW 630 – Chlorophyll *c*

Ve – Volume of extraction

4.3.8 Determination of trace metal content in sediments and water

Sediments and water samples that were collected at 5 sites during the start and end of cage experiment (Section 4.6.5 and 4.6.6) were sent to R. J. Hills Laboratories in Hamilton for analyses. For sediments, analysis for total recoverable phosphorus, total nitrogen and trace metal levels such as arsenic (As),

cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) (mg/kg dry weight) were carried out. Total nitrogen was analysed as 0.05 mg/kg (dry weight) by catalytic combustion, separation, and thermal conductivity. Total recoverable phosphorus was analysed as 40 mg/kg (dry weight) through nitric/hydrochloric acid digestion, ICP-MS (screen level) US EPA 200.2. Trace metals were analysed using nitric/hydrochloric acid digestion, ICP-MS (screen level) US EPA 200.2.

For water, analysis of total nitrogen, total ammoniacal-N, nitrite, nitrate, nitrite and nitrate, total Kjeldahl nitrogen, dissolved reactive phosphorus and total phosphorus were performed. Total nitrogen was analysed as total Kjeldahl nitrogen plus nitrate and nitrite. Total Kjeldahl nitrogen was analysed as 0.1 g/m³ by phenol/hypochlorite colorimetry. Nitrite and nitrate was analyzed as 0.002 g/m³ by automated cadmium reduction and flow injection analyser. Nitrite was analysed as 0.002 g/m³ by automated Azo dye colorimetry and flow injection analyser. Total ammoniacal-N was analysed as 0.01 g/m³ by phenol/hypochlorite colorimetry. Dissolved reactive phosphorus was analysed as 0.004 g/m³ by molybdenum blue colorimetry. Total phosphorus was analysed as 0.004 g/m³ by ascorbic acid colorimetry.

Data analyses

Average growth rates and Condition Index (CI) were analysed using Excel 2003 and PASW Statistics 18.0 software. Descriptive (mean, standard error, bar graphs and ANOVA) for average growth rates, CI, total nitrogen, total phosphorus and trace metals in sediments and water are discussed in the chapter where appropriate.

4.4 Results

4.4.1 Average growth rate of *A. crenata* at different sites

The average increase in shell length of adult *A. crenata* varied among sites (one-way ANOVA $P < 0.05$) with the rapid growth from individuals from Ferrymead (1.553 ± 0.753) and similar values ranging from 0.140 ± 0.045 (Oxidation Ponds) to 0.314 ± 0.071 (Tern Street). For the medium sized *A. crenata* shell increase varied between sites (one-way ANOVA $P < 0.001$) with the rapid growth recorded from Pleasant Point Jetty (1.107 ± 0.157) and lowest growth from Oxidation Ponds (0.489 ± 0.098). There was a significant difference in average growth of shell length for juveniles *A. crenata* among sites (one-way ANOVA $P < 0.001$) with rapid growth from Pleasant Point Jetty (2.121 ± 0.120), similar growth values ranging from 0.664 ± 0.075 (Ferrymead) to 0.681 ± 0.075 (Oxidation Ponds) (Table 4.1).

The average increase in wet weight was similar for all sites for large *A. crenata* (one-way ANOVA $P > 0.05$), however there was a significant difference for medium (one-way ANOVA $P < 0.001$) and juveniles (one-way ANOVA $P < 0.001$) amongst sites. Mud snails from Pleasant Point Jetty showed significantly higher average of wet weight for medium (0.255 ± 0.026) and juveniles (0.133 ± 0.008) compared to other sites (Table 4.2).

Table 4.1 Mean shell lengths \pm SE (mm) and growth of *A. crenata* for all size classes for Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Size class	Site	Size (mm) at initial \pm SE	Size (mm) at final \pm SE	Average growth rate of length (mm) \pm SE
Large	AV	26.327 \pm 0.482	26.771 \pm 0.499	0.510 \pm 0.153 ^{a,b}
	FM	22.508 \pm 0.597	23.877 \pm 0.705	1.553 \pm 0.753 ^c
	JT	21.427 \pm 0.531	22.546 \pm 0.643	0.813 \pm 0.19 ^{a,b}
	OP	21.443 \pm 0.378	21.546 \pm 0.493	0.140 \pm 0.045 ^a
	TS	19.811 \pm 0.448	20.105 \pm 0.420	0.314 \pm 0.071 ^a
<i>P-values</i>		0.001	0.001	0.016
Medium	AV	12.572 \pm 0.455	13.188 \pm 0.448	0.541 \pm 0.066 ^{a,b}
	FM	13.105 \pm 0.466	13.645 \pm 0.473	0.737 \pm 0.049 ^{a,b}
	JT	14.447 \pm 0.369	15.582 \pm 0.349	1.107 \pm 0.157 ^c
	OP	12.149 \pm 0.517	12.737 \pm 0.497	0.489 \pm 0.098 ^a
	TS	12.797 \pm 0.438	13.563 \pm 0.450	0.922 \pm 0.092 ^{b,c}
<i>P-values</i>		0.006	0.001	0.001
Juveniles	AV	6.302 \pm 0.990	7.089 \pm 0.200	0.825 \pm 0.072 ^{a,b}
	FM	6.670 \pm 0.143	7.343 \pm 0.146	0.664 \pm 0.075 ^a
	JT	7.003 \pm 0.107	9.131 \pm 0.116	2.121 \pm 0.120 ^c
	OP	6.733 \pm 0.098	7.586 \pm 0.102	0.681 \pm 0.075 ^a
	TS	6.741 \pm 0.123	7.932 \pm 0.131	1.073 \pm 0.109 ^b
<i>P-values</i>		0.008	0.001	0.001

Table 4.2 Mean wet weights \pm SE (g) and growth of *A. crenata* for all sizes classes for Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Size class	Site	Wet weight (g) at initial \pm SE	Wet weight (g) at final \pm SE	Average growth rate of wet weight (g) \pm SE
Large	AV	6.755 \pm 0.404	7.279 \pm 0.449	0.642 \pm 0.197
	FM	4.154 \pm 0.334	5.007 \pm 0.411	0.960 \pm 0.538
	JT	4.056 \pm 0.211	4.393 \pm 0.267	0.117 \pm 0.046
	OP	3.752 \pm 0.226	3.762 \pm 0.313	0.200 \pm 0.085
	TS	2.759 \pm 0.222	2.941 \pm 0.206	0.217 \pm 0.080
<i>P-values</i>		0.001	0.001	0.062
Medium	AV	0.689 \pm 0.657	0.873 \pm 0.074	0.176 \pm 0.022 ^{a,b}
	FM	0.865 \pm 0.089	0.980 \pm 0.097	0.160 \pm 0.016 ^a
	JT	1.043 \pm 0.067	1.292 \pm 0.080	0.255 \pm 0.026 ^b
	OP	0.637 \pm 0.075	0.778 \pm 0.081	0.130 \pm 0.018 ^a
	TS	0.754 \pm 0.069	0.876 \pm 0.080	0.148 \pm 0.020 ^a
<i>P-values</i>		0.001	0.001	0.001
Juveniles	AV	0.121 \pm 0.109	0.168 \pm 0.014	0.047 \pm 0.006 ^a
	FM	0.110 \pm 0.008	0.156 \pm 0.009	0.048 \pm 0.005 ^a
	JT	0.164 \pm 0.010	0.289 \pm 0.012	0.133 \pm 0.008 ^b
	OP	0.089 \pm 0.008	0.152 \pm 0.007	0.058 \pm 0.005 ^a
	TS	0.135 \pm 0.006	0.184 \pm 0.012	0.051 \pm 0.008 ^a
<i>P-values</i>		0.001	0.001	0.001

4.4.2 Effect of site on mortality rate

The mortality results from each of the replicate experimental plot were combined as shown in Table 4.3. Juvenile *Amphibola crenata* from Oxidation Ponds and Tern Street suffered the greatest loss (8 mudsnails for each site), at Avon as many as 7 died and at Pleasant Point Jetty only 2 died. The greatest mortality occurred in large sized mudsnails from Oxidation Ponds with 10 dead followed by Ferrymead with 9 dead. However, no dead adult mudsnail were found at Tern Street. For the medium sized class, Tern Street recorded 4 dead mudsnails and 3 dead mudsnails respectively at Ferrymead, Pleasant Point Jetty and Oxidation Ponds.

Table 4.3 Number of dead *A. crenata* found in each cage at each site after six weeks with initial populations of large ($n=15$); medium ($n=30$); and juveniles ($n=30$).

Site	Large	Medium	Juveniles
Avon	2	1	7
Ferrymead	9	3	4
Pleasant Point Jetty	8	3	2
Oxidation Ponds	10	3	8
Tern Street	0	4	8

4.4.3 Condition Index of *Amphibola crenata* at different sites

The Condition Index (CI) varied with body size and site. Figure 4.6 demonstrates that the CI of large *Amphibola crenata* was greatest at Tern Street (0.166) by a relatively large degree, compared with the lower values calculated at Avon and Ferrymead (0.094 and 0.082 respectively) (Table 4.4). There was a significant difference between the condition index of large *A. crenata* among the sites, (one-way ANOVA $F= 17.052$, $P< 0.05$) (Table 4.5).

Amongst the medium size class, those individuals sampled from Tern Street had a conspicuously higher CI of 0.225 compared with the other 3 sites. The other populations produced values of 0.123 (Avon), 0.129 (Ferrymead) and 0.136 (Pleasant Point Jetty) (Figure 4.6) (Table 4.4). There was a significance difference between the condition index of medium *A. crenata* among sites (one-way ANOVA $F= 15.898$, $P< 0.05$) (Table 4.5).

Comparison of the CI of juvenile *Amphibola crenata* across the estuary revealed Tern Street recorded the highest CI of 0.348 and Oxidation Ponds was the lowest with CI of 0.015 (Figure 4.6) (Table 4.4). There was a significance difference between the condition index of juveniles *A. crenata* among sites (one-way ANOVA $F= 21.240$, $P < 0.05$) (Table 4.5).

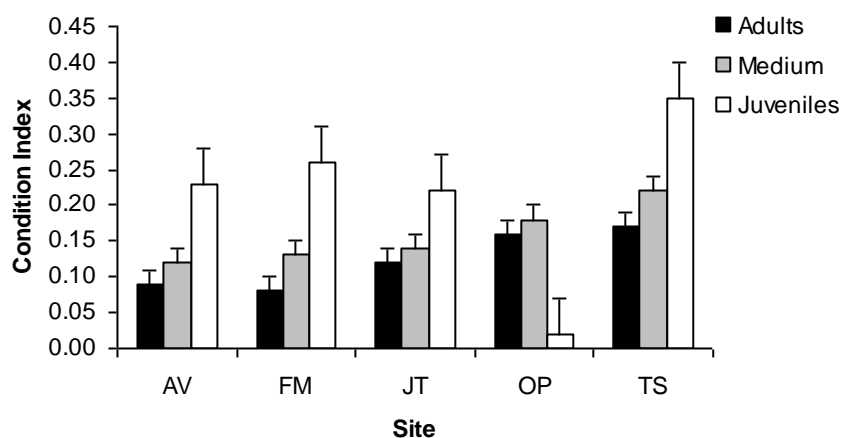


Figure 4.6 Condition index (\pm SE) of each size class of *A. crenata* at Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Table 4.4 Condition index of large, medium and juveniles *A. crenata* at Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Size class	Site	<i>n</i>	Mean (\pm SE)
Large	AV	13	0.094 (\pm 0.047) ^a
	FM	6	0.082 (\pm 0.004) ^a
	JT	7	0.119 (\pm 0.007) ^a
	OP	6	0.161 (\pm 0.024) ^b
	TS	15	0.166 (\pm 0.008) ^b
<i>P-values</i>			0.0001
Medium	AV	29	0.123 (\pm 0.006) ^a
	FM	27	0.129 (\pm 0.008) ^a
	JT	27	0.136 (\pm 0.007) ^a
	OP	27	0.181 (\pm 0.013) ^b
	TS	26	0.225 (\pm 0.017) ^c
<i>P-values</i>			0.001
Juveniles	AV	23	0.233 (\pm 0.027) ^b
	FM	26	0.265 (\pm 0.018) ^{b,c}
	JT	28	0.224 (\pm 0.023) ^b
	OP	21	0.015 (\pm 0.001) ^a
	TS	22	0.348 (\pm 0.410) ^c
<i>P-values</i>			0.001

Table 4.5 Results of analysis of variance comparing the average condition index of large, medium and juveniles *A. crenata* between sites.

Size class		Sum of squares	df	Mean squares	<i>F</i>	<i>P</i>
Large	Between sites	0.057	4	0.014	17.052	0.001
	Within sites	0.035	42	0.001		
Medium	Between sites	0.203	4	0.051	15.898	0.001
	Within sites	0.417	131	0.003		
Juveniles	Between sites	1.299	4	0.325	21.240	0.001
	Within sites	1.759	115	0.015		

4.4.4 Chlorophyll *a*, *b* and *c* content in sediment

Chlorophyll *a* content

There was high variation in average chlorophyll *a* content between the 5 sites during November 2009. The average chlorophyll *a* content varied between sites (two-way ANOVA $F= 57.1$, $P< 0.05$) (Table 4.6) which Avon (AV) produced the highest average of chlorophyll *a* content (17.95 µg/g) compared to site Oxidation Ponds (OP) (8.57 µg/g) in the experimental cages (Figure 4.7). However, the average chlorophyll *a* content did not vary between cages and there was no interaction between sites and cages affecting the chlorophyll *a* content (Table 4.6).

In December 2009, chlorophyll *a* content varied between sites (two-way ANOVA $F= 24.801$, $P< 0.05$) and cages (two-way ANOVA $F= 7.689$, $P< 0.05$) (Table 4.6) which Avon (AV) produced the highest average of chlorophyll *a* content of 15.58 µg/g in experimental cages, 18.79 µg/g in control cages and 17.48 µg/g at outside of cages (Figure 4.7). The interaction between sites and cages was also a significant factor contributing to the chlorophyll *a* content (two-way ANOVA $F= 3.001$, $P< 0.05$) (Table 4.6).

There were significant differences of chlorophyll *a* content at Avon (Mann-Whitney $P< 0.05$), Oxidation Ponds (Mann-Whitney $P< 0.05$) and Tern Street (Mann-Whitney $P< 0.05$) for the initial and final of experiment (Table 4.9).

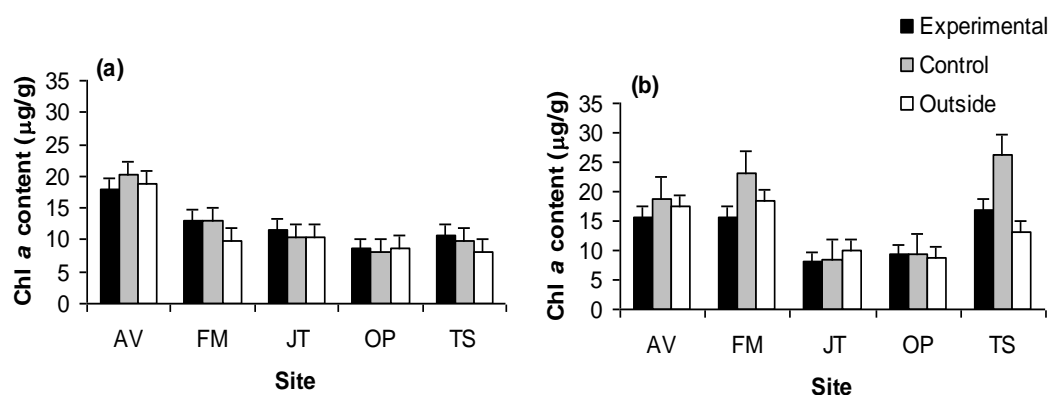


Figure 4.7 Average chlorophyll *a* content (\pm SE) in sediment at the surface of experimental cages, in the control and outside of the cages in a) November 2009, b) December 2009.

Table 4.6 Results of analysis of variance comparing the chlorophyll *a* content of sediment between sites in November and December 2009.

Time	Source of variation	SS	df	MS	<i>F</i>	<i>P-value</i>
Nov 09	Sites	624.375	4	156.094	57.080	0.000
	Cages	11.134	2	5.567	2.036	0.148
	Interaction	29.094	8	3.637	1.330	0.267
Dec 09	Sites	972.855	4	243.214	24.801	0.000
	Cages	150.808	2	75.404	7.689	0.002
	Interaction	235.456	8	29.432	3.001	0.013

4.4.5 Chlorophyll *b* content

In November 2009, the chlorophyll *b* content varied between sites (two-way ANOVA $F= 11.8$, $P < 0.05$) (Table 4.7) where Avon and Pleasant Point Jetty recorded the highest average values of chlorophyll *b* content of $1.46 \mu\text{g/g}$ and $1.41 \mu\text{g/g}$ respectively in the experimental cages (Figure 4.8). At Tern Street the quantity of chlorophyll *b* content was below zero and thus was included in the graph. However, there was no effect on cages and interaction between sites and cages did not contribute on the response of chlorophyll *b* content (Table 4.7).

In December 2009, the chlorophyll *b* content varied among sites (two-way ANOVA $F= 21.9$, $P < 0.05$) (Table 4.7) where Avon and Pleasant Point Jetty

produced the highest average of chlorophyll *b* content of 1.94 $\mu\text{g/g}$ and 1.93 $\mu\text{g/g}$ respectively in the experimental cages (Figure 4.8). However, the average chlorophyll *b* content did not vary between cages and the interaction between sites and cages were also not affecting the chlorophyll *b* content (Table 4.7).

There were significant differences in chlorophyll *b* content at all sites (Mann-Whitney $P < 0.05$) at the start and end of the experiment (Table 4.9).

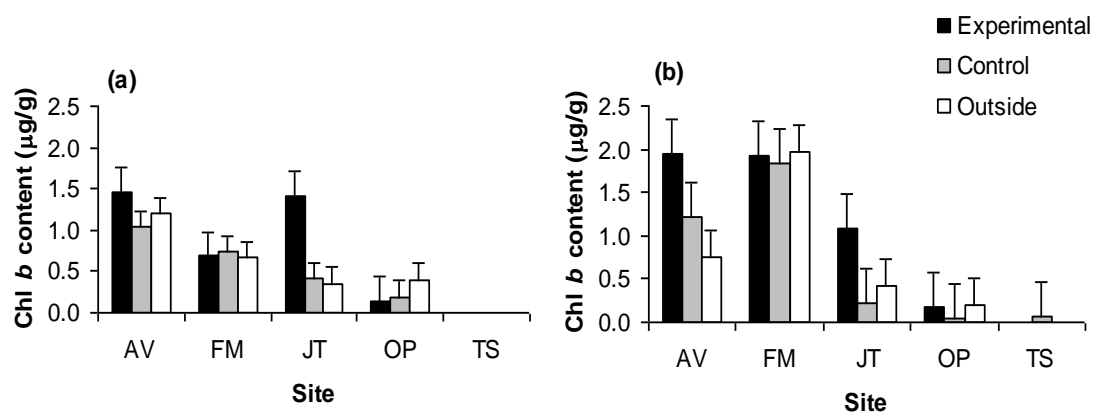


Figure 4.8 Average chlorophyll *b* content (\pm SE) in sediment at the surface of experimental cages, in the control and outside of the cages in a) November 2009, b) December 2009.

Table 4.7 Results of analysis of variance comparing the chlorophyll *b* content of sediment between sites in November and December 2009.

Time	Source of variation	SS	df	MS	<i>F</i>	<i>P-value</i>
Nov 09	Sites	10.987	4	2.747	11.806	0.001
	Cages	0.522	2	0.261	1.122	0.339
	Interaction	2.096	8	0.262	1.126	0.375
Dec 09	Sites	24.811	4	6.203	21.889	0.001
	Cages	1.482	2	0.741	2.616	0.090
	Interaction	2.161	8	0.270	0.953	0.489

4.4.6 Chlorophyll *c* content

In November 2009, the chlorophyll *c* content varied between sites (two-way ANOVA $F=27.4$, $P<0.05$) (Table 4.8) which Avon recorded the highest average of chlorophyll *c* content of $7.17 \mu\text{g/g}$ while Oxidation Ponds was the lowest of $3.27 \mu\text{g/g}$ in chlorophyll *c* content in the experimental cages (Figure 4.9). There was no effect on cages and interaction between sites and cages did not contribute on the response of chlorophyll *c* content (Table 4.8).

In December 2009, chlorophyll *c* content varied between sites (two-way ANOVA $F=33.8$, $P<0.05$) and cages (two-way ANOVA $F=9.2$, $P<0.05$) (Table 4.8). The highest chlorophyll *c* content was found at Ferrymead ($10.52 \mu\text{g/g}$) and Tern Street ($10.13 \mu\text{g/g}$) in control cages (Figure 4.9). The interaction between sites and cages were also a significant factor contributing to the response of chlorophyll *c* content (two-way ANOVA $F=4.8$, $P<0.05$) (Table 4.8).

There were significant differences at Ferrymead, Pleasant Point Jetty, Oxidation Ponds and Tern Street (Mann-Whitney $P<0.05$) on the level of chlorophyll *c* content (Table 4.9).

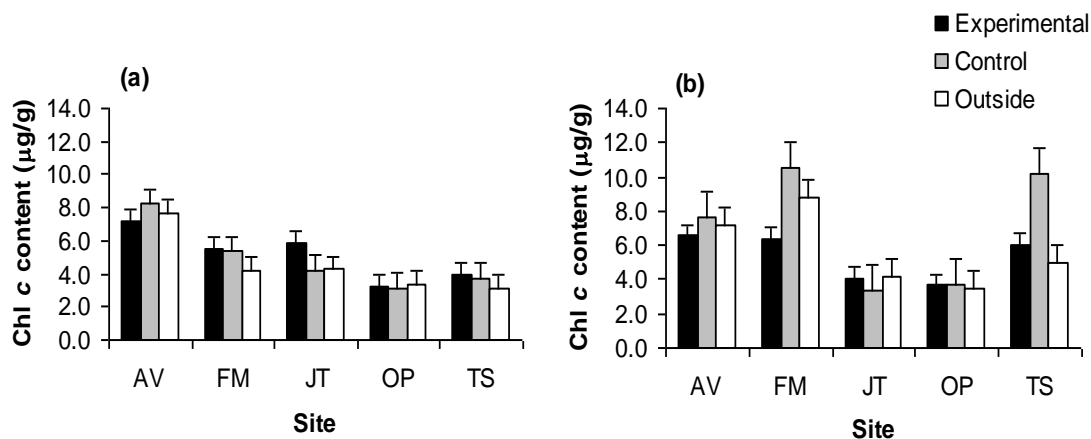


Figure 4.9 Average chlorophyll *c* content (\pm SE) in sediment at the surface of experimental cages, in the control and outside of the cages in a) November 2009, b) December 2009.

Table 4.8 Results of analysis of variance comparing the chlorophyll *c* content of sediment between sites in November and December 2009.

Time	Source of variation	SS	df	MS	F	P-value
Nov 09	Sites	108.535	4	27.134	27.386	0.001
	Cages	3.057	2	1.529	1.543	0.230
	Interaction	8.622	8	1.078	1.088	0.398
Dec 09	Sites	173.002	4	43.250	33.841	0.001
	Cages	23.392	2	11.696	9.152	0.001
	Interaction	48.813	8	6.102	4.774	0.001

Table 4.9 Mann-Whitney values comparing the chlorophyll *a*, *b* and *c* content of sediment between the start and end of the field experiment at Avon (AV); Ferrymead (FM); Pleasant Point Jetty (JT); Oxidation Ponds (OP); and Tern Street (TS).

Chlorophyll	Site	Mann-Whitney U	Wilcoxon W	Z	P
Chlorophyll <i>a</i>	AV	0.000	45.000	-3.576	0.001
	FM	36.000	81.000	-0.397	0.691
	JT	32.000	77.000	-0.385	0.700
	OP	9.000	54.000	-2.782	0.005
	TS	2.000	47.000	-3.400	0.001
Chlorophyll <i>b</i>	AV	17.000	62.000	-2.075	0.038
	FM	5.500	50.500	-3.092	0.002
	JT	6.000	51.000	-3.051	0.002
	OP	2.000	47.000	-3.401	0.001
	TS	0.000	45.000	-3.473	0.001
Chlorophyll <i>c</i>	AV	27.000	72.000	-1.192	0.233
	FM	9.000	54.000	-2.782	0.005
	JT	7.000	52.000	-2.958	0.003
	OP	0.000	45.000	-3.578	0.001
	TS	2.000	47.000	-3.400	0.001

4.4.7 Total phosphorus, total nitrogen and trace metals in sediments and water

Due to the insufficient resources for this research, only one sample from the experimental mudsnail plots from each site from the start and end of the experiment were sent to the Hills Laboratories for analysis. Thus statistical analyses were not undertaken as the findings were not adequate to show whether the levels of total phosphorus, total nitrogen and trace metals in sediments and water affected the growth rates and condition index of *A. crenata*. The only trends discussed in this section were the variation between the start and end of the experiment and the site differences.

Levels of total phosphorus in the sediments were highest at Avon and Pleasant Point Jetty and similar at Pleasant Point Jetty, Oxidation Ponds and Tern Street. Levels of total nitrogen were higher in December rather than November 2009 except at Ferrymead. Avon showed the highest total nitrogen compared to other sites. Cadmium had the lowest trace metal concentration found among sites and zinc was the highest trace metal found (Figure 4.10).

Levels of total phosphorus, total nitrogen, total ammoniacal, total kjeldahl nitrogen and dissolved reactive phosphorus in the water were highest in the Oxidation Ponds in December 2009. Nitrites were low in all samples while levels total kjeldahl nitrogen was quite high in all water samples (Figure 4.11).

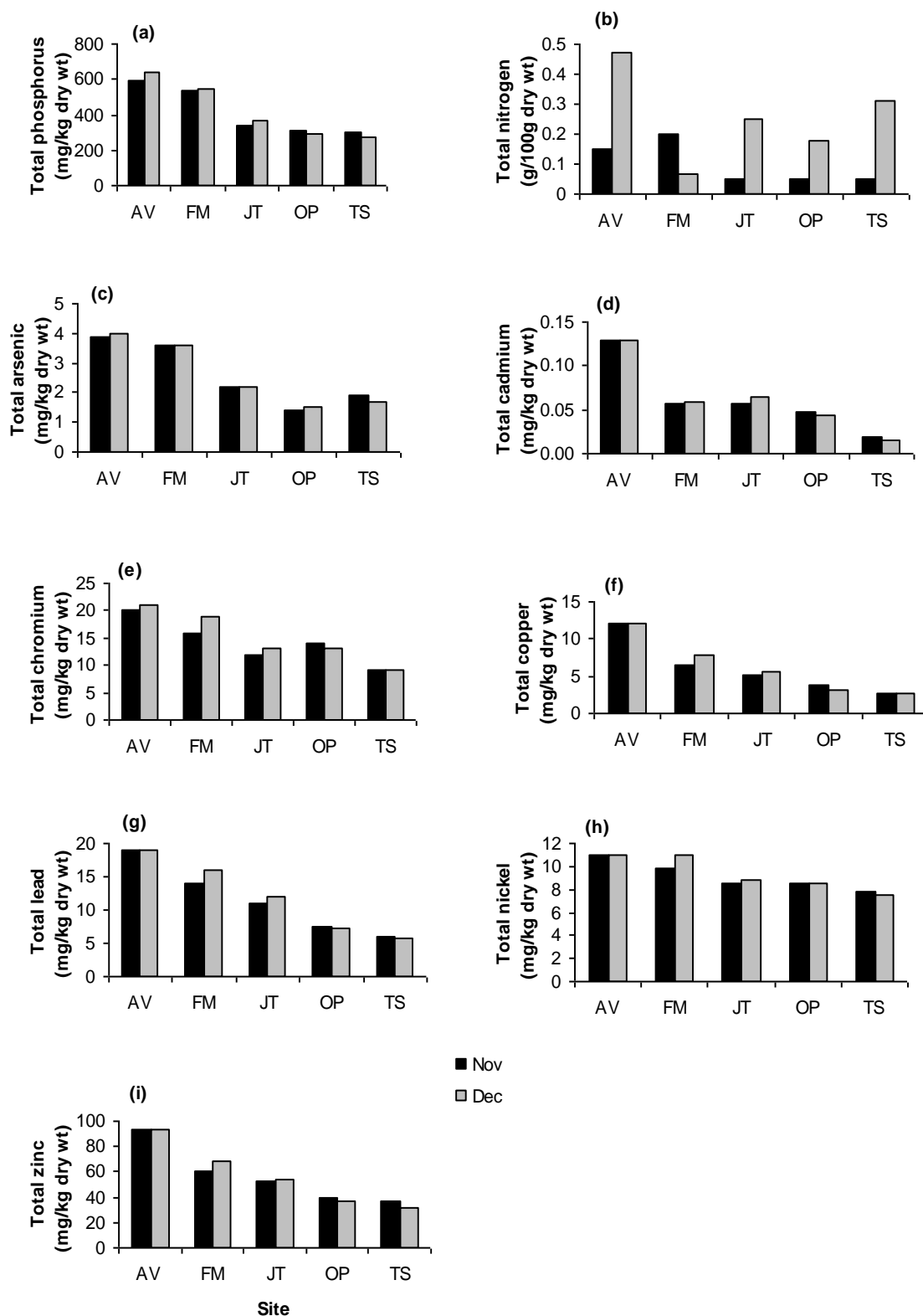


Figure 4.10 Levels of nutrients and trace metals in the sediments; a) total phosphorus; b) total nitrogen; c) arsenic; d) cadmium; e) chromium; f) copper; g) lead; h) nickel; and i) zinc in November and December 2009 at Avon (AV), Ferrymead (FM), Pleasant Point Jetty (JT), Oxidation Ponds (OP); and Tern Street (TS).

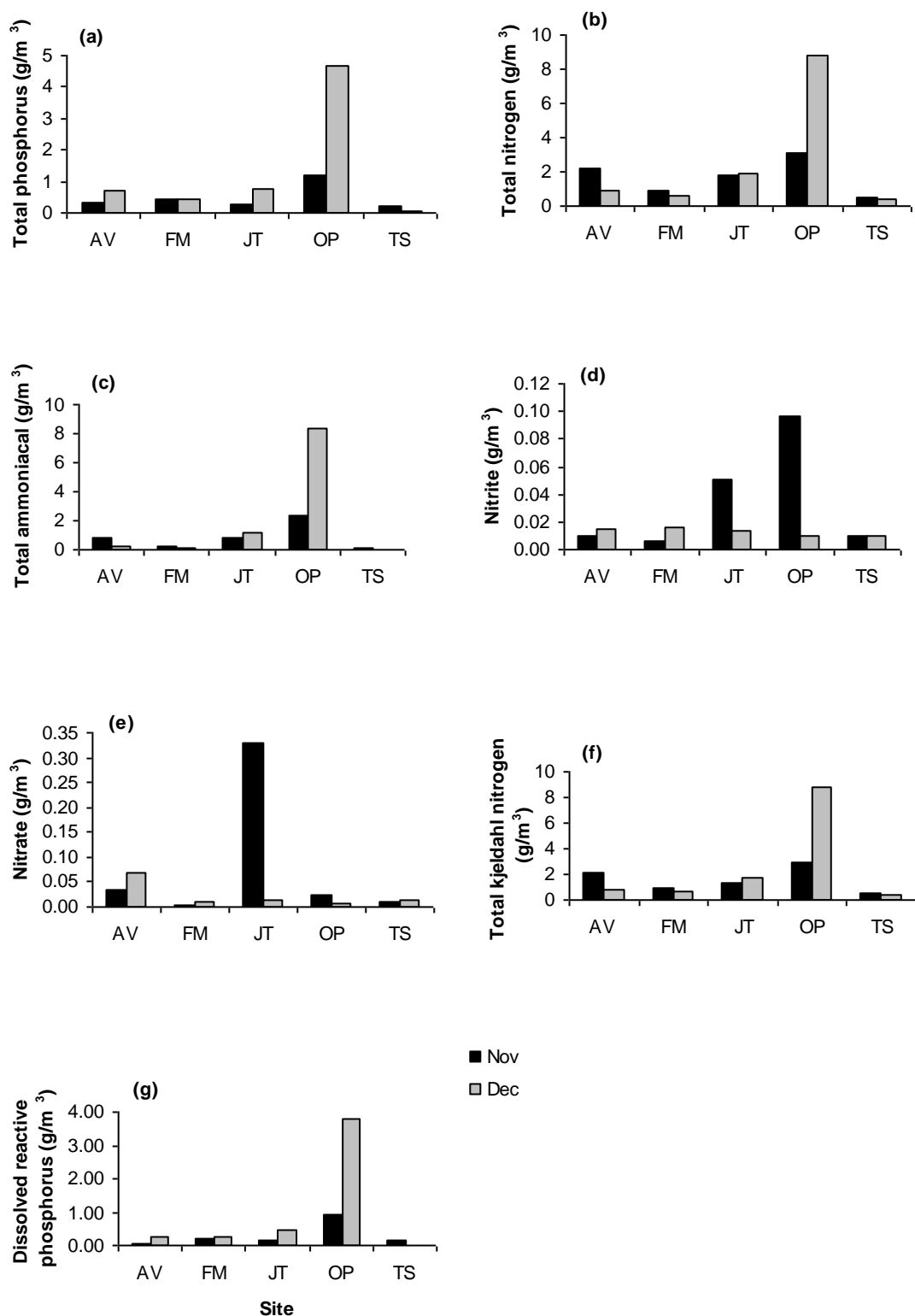


Figure 4.11 Nutrient levels in the water at different sites; a) total phosphorus; b) total nitrogen; c) total ammoniacal; d) nitrite; e) nitrate; f) total kjeldahl nitrogen; and g) dissolved reactive phosphorus in November and December 2009 at site Avon (AV), Ferrymead (FM), Pleasant Point Jetty (JT), Oxidation Ponds (OP); and Tern Street (TS).

4.5 Discussion

Growth rates and condition

A number of studies have recorded spatial and temporal differences in growth for mollusc populations. Shell growth of adult *A. crenata* was highest at the Ferrymead site (1.553 ± 0.753) which was relatively close to the estuary mouth. At this site the sediment consisted of fine silts or clay. In the present study, correlation between sediment types and growth rate was not undertaken however there are examples from the literature supporting the relationships between these factors. For hydrobiid gastropods the silt-clay content of the sediment was positively correlated with body size and population density (Newell 1965; Chatfield 1972; Fish and Fish 1974). In *Hydrobia truncata* collected from three locations (Flax Ponds; Setauket Harbour; and West Meadow Beach) in New York individuals were greater from muddy rather than in sandy habitats (Forbes and Lopez 1990). In the present study however, the average wet weight did not vary amongst sites and this finding contrasts with a study by Morrissey (1990), where the average body weight of *Hydrobia ulvae* (Pennant) living on fine mud was consistently higher than that of the same species living on muddy sand.

The medium and juvenile *A. crenata* collected from Pleasant Point Jetty had the most rapid growth rates. This can be explained by the higher quality of the food resources. Chlorophyll *a*, *b* and *c* in the experimental cages at the beginning of the experiment provided a good food source for the growth of *A. crenata*. By the end of the experiment at this site, the quantity of chlorophyll on the sediment had decreased. Food resources are vital components of a habitat and they can affect organisms through direct or indirect impacts in the food chain. This can be important in stressful, chronic and low-level disturbances due to the long-term effluent discharge in estuarine ecosystems (Bortone 2005).

The growth rate of juvenile *A. crenata* outside the Oxidation Ponds was the lowest of all the sites. In the experimental cages chlorophyll *a*, *b* and *c* increased from the start to the end of the experiment and it can be concluded that *A. crenata* did not feed on the chlorophyll in the sediment surface. Even though food resources were available for these gastropods, the quality and quantity are crucial

and maybe the food was nutritionally unsuitable for juveniles *A. crenata* to feed on. Anoxic conditions at this site may have modified the bacterial food assemblages and stress conditions in the cages could also alter the grazing activity of juvenile *A. crenata*. Habitat modifications created by the cages are often neglected in cage experiments (Hulberg and Oliver 1980; McClatchie *et al.* 1982). Water flow can interrupt the enclosure and modify the hydrographic movement (Hulberg and Oliver 1980). Plant and animals can also affect the cages depending on the degree of water movement, thus alter the depositional environment and leading to the accumulation of finer sediments within enclosures. However, these problems were minimized where possible by checking the cages every week and by the removal of algae or animals that were trapped on the outside of the cages.

In invertebrates the condition index often varies with location. The CI for *A. crenata* in the present study was highest at Tern Street for large and medium sized classes but lowest at Ferrymead (large) and Pleasant Point Jetty (medium individuals). Sediment composition can influence the CI of organisms at particular sites. The CI, measured by the ratio of wet tissue weight to shell weight of *Rangia cuneata* (bivalve) was higher in sand substrata than in mud with a high organic content (Tenore *et al.* 1968).

Differences of sediments explained differences in CI, high values from Tern Street (firm mud/sand) and lower values from Ferrymead (fine silts/clay); and Pleasant Point Jetty (fine mud/sand). The CI of juvenile *A. crenata* at the Oxidation Ponds was the poorest (0.015) compared to other sites. The short-lived anoxic condition developed at this site was most likely due to the release of effluent in the estuary. The higher mortality rate of juveniles at this site also suggests that this is the least suitable site for growth and survival.

The chlorophyll levels recorded in the experimental cages at Ferrymead, Oxidation Ponds and Tern Street were higher at the end of the experiment compared to the initial values. It was thought that in the experimental cages, the chlorophyll levels would decrease by the end the experiment due to the feeding activity of *A. crenata* on the sediment surface. Sediment porosity and drainage can influence the time available for feeding in intertidal habitats (Forbes and Lopez

1990). *Hydrobia truncata* retreats into its shell when the sediment loses moisture during the exposure at low tide (Forbes 1988 cited in Forbes and Lopez 1990). Sandy sediments generally have greater porosity and in *H. truncata* there was decreased feeding activity in sand compared to muddy sediments. Similar findings were not found in the present study where Ferrymead sediment consisted of fine silts or clay and Oxidation Ponds and Tern Street consisted of firm mud or sand.

In the present study the chlorophyll *a* content varied between sites and cages at the end of the experiment in December 2009 (two-way ANOVA $F=7.689$, $P<0.05$). Also, the levels of chlorophyll *a* content were higher in control cages than in the experimental cages. Chlorophyll *a* content can act as an index of photosynthetic potential and biomass of benthic microalgae (MacIntyre *et al.* 1996). In McClatchie *et al.* (1982) in the Avon-Heathcote Estuary and Juniper (1982) in Delaware Inlet, New Zealand it was found that primary production and diatom densities were higher in cages that consists no mudsnails compared to cages that had double density of *A. crenata*. Higher densities of the deposit-feeding gastropod, *Ilyanassa obsoleta* can reduce the growth of diatom (Pace *et al.* 1979 cited in Juniper 1987a). Increasing snail density and grazing pressure can also increase the number of diatom taxa significantly (McClatchie *et al.* 1982). However, it can also reduce benthic microalgae production (Juniper 1982).

Benthic microalgae biomass is often more obvious on sandy sediments (Lever and Valiela 2005) due to higher permeability, porosity and flow velocities. Benthic microalgae biomass has been measured in eutrophic Bodden estuaries of the Baltic Sea. Here the benthic microalgae's contribution to total productivity was higher at sandy Kirr Bucht were (37%) compared to muddy Rassower Strom (30%) (Gerbersdorf *et al.* 2005).

Nutrients and trace metals in the sediments and water

Nutrient levels in estuaries receiving sewage and other effluent are often high. One site close to the Avon River had the highest levels of total phosphorus, total nitrogen and trace elements such as arsenic, cadmium, chromium, copper, lead, nickel and zinc. This may be due to the location of the site which is situated

at the river mouth of the Avon River and highly variable discharges and associated contaminants inputs released by the river (Knox and Kilner 1973). The sediments at this site consist of very soft mud allowing the total phosphorus, nitrogen and trace metals to accumulate in the sediments. In estuaries around the world, the accumulation of fine suspended sediments and the mixing of fresh and marine water acts as a trap for trace metals (Bonnerie *et al.* 1994, Fernandez Caliani *et al.*; 1997). Concentrations of trace metals are associated with sediment particle size and higher concentrations of trace metals are trapped in finer particles (Phillips and Rainbow 1994). Higher concentrations of copper and zinc in the sediments increased with the percentage of smaller sediment particles (Milne 1998).

Cadmium was the element that occurred in lowest concentrations while zinc had the highest trace metal concentrations detected in the present study. This was similar to results from the Auckland region (Stewart 2005). Trace metals had decreased significantly at all sites sampled in the present study compared to earlier studies by Robb (1988). Copper and zinc level at outside of the waste water treatments pond outlets were 24.6 mg/kg and 135.2 mg/kg in 1988, however the levels dropped to 3.8 mg/kg (copper) and 39 mg/kg (zinc) in the present study. Since 1962, the treatment plant has been upgraded substantially and in 2010 the waste pipe discharging into the estuary was replaced by a 3 km-long underground pipe that now discharges directly into the ocean. There has been a dramatic reduction in the amount of fresh water as well as inorganic, organic nutrients and trace metals discharged into the estuary.

One reason why the levels of trace metals might differ in the present study compared to 1988 study may due to the different analytical techniques that were used. Robb used a Perkin Elmer 3110 flame atomic adsorption spectrometer, after extraction with a mixture of 4% nitric acid and 2.5% prechloric acid for two hours at 90°C. The same analytical technique was also used by Nipper *et al.* in 1997. He found higher level of copper (16.6 mg/kg) and zinc (89 mg/kg) compared to the present study. Nipper *et al.* (1997) found 10 mg/kg of copper and 90.2 mg/kg of zinc, however these levels had dropped to 6.4 mg/kg of copper and 60 mg/kg of zinc in the present study.

Nutrient levels, total phosphorus (4.7 g/m^3), nitrogen (8.8 g/m^3), ammoniacal (8.4 g/m^3), kjeldahl nitrogen (8.8 g/m^3) and dissolved reactive phosphorus (3.8 g/m^3) in the water were highest in the Oxidation Ponds in December 2009 compared to other sites. Bolton-Ritchie and Main (2005) study also found high total phosphorus (6.95 g/m^3), nitrogen (33.8 g/m^3) and dissolved reactive phosphorus (5.5 g/m^3) in the water compared to present study. Before 2010 about 90% of organic nitrogen and 98% of organic phosphorus, sediments, heavy metals, organic matter and contaminants, pathogens and freshwater were released into the estuary from the water treatment plant (URS, 2001). Dissolved inorganic nitrogen will drop significantly upon the wastewater diversion to the ocean (Walrond 2007). Loss of the primary production will also be expected due to the sewage diversion and this could bring long term ecological consequences for population structures, communities and the food webs in the estuary (Kennish 1992).

Replication in experimental design is very important in order to determine accurate and statistically valid results. It is important because it adds information about the reliability of the conclusions or estimates to be drawn from the data. Therefore, the samples must consist of measurements from a number of samples (Zar 1999). It would also useful to use multivariate analysis to find interrelationships between environmental parameters such as chlorophyll content, total nitrogen, phosphorus and trace metals with the growth rates of each size class of *A. crenata*.

4.6 Conclusions

As in many other studies it was found that the growth rate and condition index of *A. crenata* varied between size classes and sites. This was most likely due to a combination of factors including food quality, contaminant levels and sediment conditions. The Condition Index (CI) also varied between size classes and site and was higher in sandy substrates than muddy sediment types. The chlorophyll *a* content of the sediment was higher in control rather than experimental cages and primary production and diatom densities were higher in

cages with no mudsnails. Factors such as sediment porosity and drainage decreased the feeding activity of *A. crenata*.

Total phosphorus, nitrogen and trace metals in sediments were higher in Avon due to the location of the site at the Avon river mouth which highly variable discharged and associated contaminants input that were released by the river. The sediments which consist of very soft mud allow the greatest level on the accumulation of trace metals in the sediments compared to other sites. Zinc was found in highest concentrations while cadmium was the lowest trace metals detected across all sites of the Avon-Heathcote Estuary.

Total phosphorus, nitrogen, and trace metals in the water were higher at the most polluted site which is Oxidation Ponds. This site was the main point source of nutrients in the Avon-Heathcote Estuary. The diversion of effluent from the Avon-Heathcote Estuary is expected to reduce algal productivity and decrease mudsnail biomass. It is expected that there would be improved CI of *A. crenata* at the most polluted site. However, the change in nutrient enrichment in the estuary may result in shifts in the population structure, density and distributions of *A. crenata*.

Chapter 5: GENERAL DISCUSSION

5.1 Summary of major findings

Estuaries provide numerous benefits for humans, for transportation, recreation, fish and shellfish harvesting. Many large cities are located on estuaries and they receive discharges from municipal, industrial and agricultural waste from coastal urban development. Today only a small proportion of estuaries remain in a pristine condition (Robertson *et al.* 2002). Worldwide, estuaries are under threat due to anthropogenic eutrophication, particularly from the direct discharge of treated sewage, which is high in macronutrients such as nitrogen and phosphorus (Nixon 1995; Richardson and Jorgensen 1996).

Many studies have been undertaken on the macrofaunal densities, distributions; benthic microalgae, macroalgal abundance, productivity; and sediment biogeochemistry of the Avon-Heathcote Estuary (Marsden and Knox 2008). The importance of regular monitoring and baseline surveys have been stressed by Raffaelli (1999, p. 450); “In order to make a persuasive case for remedial measures, especially in the early stages of eutrophication... a before and after comparison is likely to be more convincing for managers and has greater relevance for addressing the problems of a particular estuary”.

This thesis investigated the current distribution and abundance of the indicator species *Amphibola crenata* in the Avon-Heathcote Estuary prior to the diversion of treated sewage through a three km ocean pipeline into the ocean. *A. crenata* was widely distributed throughout the estuary; its highest densities were found near to Sandy Point some 66.7 meters from the Pleasant Point Jetty, with more than 151 individuals/m². Individuals occurred within 200 m of the edge of the estuary where densities ranged from 1–150 individuals/m². However, no mud snails were found in samples collected along the Causeway, McCormacks Bay, Beachville Road and on the sand banks in the centre of the Avon-Heathcote Estuary. The highest mud snail biomass occurred at the Linwood site, west of Mt. Pleasant Yacht Club and 200 meters from the edge of estuary at Pleasant Point Jetty with more than 4.1 g/m². When mud snails were present the biomass ranged between 0.1–4.0 g/m² within 200 meters of the estuary edge. There was a positive

correlation between density and biomass and a negative correlation with distance from the edge of the estuary. *A. crenata* density and biomass were also negatively correlated with salinity. Positive correlations occurred between the percentage of pore water in sediments and the percent organic matter in the sediments.

Winter and summer populations were sampled to determine the seasonal abundance of *A. crenata* at selected sites. Densities varied amongst sites and tidal levels in winter and summer. High densities were found at one known contaminated site, Oxidation Ponds, with 28 individuals/m² in winter and 60 individuals/m² in summer. At all five sites, higher densities of *A. crenata* were found at the high-tide level than the mid- and low-tide levels. The size of *A. crenata* increased with water depth towards the middle of the inlet. Juvenile *A. crenata* favoured the high-tide level rather than mid- or low-tide levels, probably due to the finer sediments that are most suitable for settlement. Medium and adult *A. crenata* commonly inhabit the mid- and low-tide levels due to their high tolerance to changing salinity. There was a significant correlation between the dry weight and shell length of *A. crenata* at all sites in both winter and summer samples. However, there was no significant difference in the weight relationships between sites. The percent organic content in the sediments varied between sites in winter but not in summer. Differences in the sediment composition amongst sites might explain the differences of organic content in the sediments. The porosity of the sediments was significantly higher at high- and mid-tide level during winter and summer.

Field cage experiments were used to investigate the growth and condition of *A. crenata* at five selected sites over summer. The benthic microalgae which form part of the food source for *A. crenata* were also quantified together with changes in nutrient levels. Over a six week period, shell growth of adult, medium and juvenile *A. crenata* varied amongst sites. Although the wet weight of adults did not change significantly, there were differences in weight gain of medium and juveniles from the different sites. In the present study, food resources were estimated from chlorophyll *a*, *b* and *c* levels in the upper 2 mm of sediment surface. The quality and quantity of the food resource were the main factors

contributing to the rapid growth rates of *A. crenata*. Mud snail condition varied with body size and sites, with lowest values recorded from adults at Ferrymead, medium individuals from Pleasant Point Jetty and juveniles from the Oxidation Ponds. One of the sites (Avon) had high sediment concentrations of total phosphorus, total nitrogen and trace elements including arsenic, cadmium, chromium, copper, lead, nickel and zinc. This is due to the location of the Avon site which is situated at the river mouth. Zinc was the trace metal found in the highest concentrations while cadmium was the lowest trace metals detected in the sediments. Levels of trace metals were lower in the present study than in the 1988 study by Robb. Levels of total phosphorus, nitrogen, ammoniacal, kjeldahl nitrogen and dissolved reactive phosphorus in the water were higher at the Oxidation Pond site which was close to the main discharge site from the treatment plant into the Avon-Heathcote Estuary.

5.2 Critique of methods

Cages used in field experiments have some limitations that may affect the growth rates and condition of molluscs amongst sites. The reduction of water flow, alteration of organisms' behaviour, increased sedimentation and shading are problems previously encountered by scientists using cages in manipulative field experiments (Miller and Gaylord 2007). Nutrient enrichment that occurred in Masonboro Inlet, North Carolina increased the benthic microalgae biomass in cages containing deposit feeders and their predators (Posey *et al.* 1995). Studies by Como *et al.* (2006) on a micro-tidal mudflat that used similar cages to those used in the present study, found that benthic microalgae biomass increased within the cages compared to outside regardless of cage's mesh size. In the present study, if the cages containing *A. crenata* trapped fine sediment then this could affect the food availability. In addition the cages may protect individuals from bird predation and this might also influence the growth rates and the condition index.

5.3 Monitoring / Indicators

The present study has shown that benthic invertebrates such as *A. crenata* can be useful indicators of estuarine health. This species is likely to be a good indicator of changes in nutrient conditions within estuarine ecosystems. The density and population structure of *A. crenata* responded to a number of environmental variables. It is important to fill any gaps in our understanding of how species respond to environmental variables. The interaction between habitat and environmental heterogeneity is crucial as species-environment correlations are important tools for ecology and conservation (Rossi *et al.* 2007). Monitoring species that are ecologically and commercially important in the receiving environment should be chosen precisely (Lam and Gray 2001). In the present study, *A. crenata* was sampled throughout the estuary and seasonally during winter and summer at selected sites. By extending the seasonal survey it may be possible to understand the relationships between *A. crenata* populations, environmental variables and other species. In the Bay of Fundy, Canada for example the presence of mudsnails, *Ilyanassa obsoleta* in July could reduce recruitment of the amphipod *Corophium volutator*, and therefore reduce the prey availability for shorebirds arriving later in the summer (Hamilton *et al.* 2003). The authors explained that a better understanding of shorebird habitat use is needed and stressed the importance of conserving particular mudflats in the future. The spatial and temporal variations of *A. crenata* populations could be determined by monitoring the Avon-Heathcote Estuary in regular intervals over a number of years.

5.4 Management Strategies

New Zealand estuaries are exposed to pressures and impacts as a result of urban coastal development. The New Zealand's Resource Management Act, and the National Protocol: Estuarine Environmental Assessment and Monitoring (Ministry for the Environment) provide strategies nationally for estuarine managements (Robertson *et al.* 2002). The Avon-Heathcote Estuary is a bar-built, shallow, well-flushed estuary, dominated by mud or sand habitat which is typical

of many estuaries found in New Zealand. All major intertidal habitats are present and its conditions have been modified by long-term effluent input (Robertson *et al.* 2002). Due to the cessation of wastewater discharge into the Avon-Heathcote Estuary, researchers anticipate that there will be changes in the geophysical, chemical and biological communities of the estuary.

The endemic deposit feeding mudsnail *A. crenata* is widely distributed across a range of geographically diverse estuaries in New Zealand. It is a suitable species to use as an ecological indicator to assess estuarine health. The survey work done as part of this research could form part of a standardised monitoring procedure which could be applied effectively in different regions of New Zealand. In addition, focusing on a surface feeding benthic species in the intertidal zone allows an evaluation of the pollution effects. The retentive and cumulative characteristics of the sediments and biota can provide stable indices of temporal variability in contaminants (Robertson *et al.* 2002). In the Avon-Heathcote Estuary, if contaminant levels change due to the release of treated waste water into the sea, then repeat benthic monitoring within the estuary will detect changes in population structure and abundance. This monitoring programme could be repeated at five year intervals by selecting few indicator species from the flora and fauna of the estuary. Any changes from the baseline study undertaken in the summer of 2009 could also be identified. Other physical and chemical characteristics such as temperature, salinity and sediments types of the Avon-Heathcote Estuary could also be included in the National Protocol trial. In assessing estuarine health, rapid and straightforward tools are crucial. It is also important to involve stake holders and managers in monitoring programmes so that the estuarine resources are protected for the benefit of everyone.

5.5 Conclusions

Spatial and temporal variations in environmental factors, for example temperature, sediment particle size, water depth, quality and salinity are important components of the estuarine ecosystems (Thrush *et al.* 1997). They determine how populations are distributed along gradients (Edgar and Barret 2002), and physical

factors such as habitat characteristics and substrate may also affect the spatial patterns of estuarine species (Hewitt *et al.* 1997). The diversity and abundance of marine benthic organisms can be threatened by human activities which can also interfere with vital ecological processes (Lindergarth and Underwood 1999). The diversion of treated sewage away from the Avon-Heathcote Estuary may have immediate and gradual effects on the estuarine ecosystems. After the sewage discharge was removed away from the Mondego Estuary, 200 km northern of Lisbon in Portugal, the sediment structure improved and there was faunal recolonisation of a previously eutrophic zone in the estuary (Teixeira *et al.* 2008). In the Firth of Clyde, Scotland disturbance indicators were still present after fourteen years of cessation of sewage disposal (Moore and Rodger 1991 cited in Smith and Shackley 2006), whilst macrobenthos of the fjords in Sweden took five years to recover from the cessation effects (Rosenberg 1973 cited in Smith and Shackley 2006). From these studies it is clear that, for effective management, there is a need for long term monitoring studies to follow the recovery from eutrophication. The spatial patterns of marine benthic organisms are important and these can be done by sampling patterns of natural scales of variation (Cole *et al.* 2000).

A. crenata populations in the present research have shown strong correlations with environmental variables and this can be used for management and conservation. Field experiments have demonstrated that quality and quantity of food resources in terms of chlorophyll *a*, *b*, and *c* are important in determining the survival of juvenile *A. crenata*. Currently, scientists at the National Institute of Water and Atmospheric Research (NIWA) are carrying out stable isotope studies to identify nutrient uptake within the estuarine food web. Similar studies have been undertaken in the Matapouri Estuary, North Island of New Zealand where the diet composition of two grazing snail species was compared (Alfaro *et al.* 2006). It would therefore be useful to include *A. crenata* as a key indicator species in the Avon-Heathcote isotope study so that pre- and post- diversion research aims and objectives can be broadened.

The research presented in this thesis provides a starting point in our understanding of the effects and implications of the diversion of sewage on the distribution and abundance of a key gastropod. Future research could also include other indicator species and other estuaries and bays of the New Zealand coastline. With improved water quality and changes in sediment properties and hydrology, it might be expected that the fauna of the Avon-Heathcote Estuary would change. In some locations there might be a change from communities dominated by deposit-feeding species to suspension-feeding organisms like cockles. The Avon-Heathcote Estuary therefore provides researchers with an ideal field laboratory to test and understand the effects and interactions of nutrients, contaminants and estuarine organisms.

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Appendix 1: The GPS coordinates for sites used in the seasonal study.

Site	Level	Left	Right
Avon	High-tide	E2488225, N5741974	E2488197, N5741964
	Mid-tide	E2488196, N5741973	E2488172, N5741952
	Low-tide	n/a	n/a
Ferrymead	High-tide	E2486370, N5738480	E2486350, N5738456
	Mid-tide	E2486390, N5738487	E2486373, N5738463
	Low-tide	E2486428, N5738447	E2486417, N5738454
Pleasant Point Jetty	High-tide	E2488242, N5741581	E2488232, N5741557
	Mid-tide	E2488315, N5741587	E2488309, N5741565
	Low-tide	E2488413, N5741538	E2488405, N5741548
Oxidation Ponds	High-tide	E2487359, N5740502	E2487371, N5740529
	Mid-tide	E2487402, N5740484	E2487418, N5740512
	Low-tide	E2489614, N5739462	E2487491, N5740466
Tern Street	High-tide	E2486414, N5738421	E2489499, N5739583
	Mid-tide	E2489423, N5739547	E2489418, N5739576
	Low-tide	E2489350, N5739535	E2489348, N5739505

Appendix 2: Unique codes for *A. crenata* used in cage experiment.

Size Class	Mudsnail No.	Colors
Large (> 18.0 mm)	1	1 dot blue
	2	2 dots blue
	3	3 dots blue
	4	4 dots blue
	5	5 dots blue
Medium (8.0-17.9 mm)	1	1 dot pink
	2	2 dots pink
	3	3 dots pink
	4	4 dots pink
	5	5 dots pink
	6	1 dot blue + 1 dot pink
	7	1 dot blue + 2 dots pink
	8	1 dot blue + 3 dots pink
	9	1 dot blue + 4 dots pink
	10	1 dot blue + 5 dots pink
Small (< 7.9 mm)	1	1 dot green
	2	2 dots green
	3	3 dots green
	4	4 dots green
	5	5 dots green
	6	1 dot blue + 1 dot green
	7	1 dot blue + 2 dots green
	8	1 dot blue + 3 dots green
	9	1 dot blue + 4 dots green
	10	1 dot blue + 5 dots green